

Defending the high frontier

President Reagan's call on the scientific community to construct defences against hostile nuclear weapons may not be pure science fiction. But it is a dangerous line to follow.

HOW can the development of a defensive weapon be counted as an aggressive act? Twenty years ago, when the two superpowers based their strategic planning on the doctrine called "mutually assured destruction" (acronym MAD), it was briskly argued that protecting the civilian population against nuclear attack by building shelters would seem to the other fellow to be a hostile act, upsetting the strategic balance and perhaps even inviting a pre-emptive strike. In the event, of course, effective shelters were discovered to be physically unfeasible and financially out of bounds. And then, in any case, military technology moved on, nuclear missiles became more accurate, more powerful and more numerous, each more often than not carrying several warheads. President Reagan has been saying since he took office that this new and more complicated strategic balance has now been upset by the speed with which the Soviet Union has been improving its strategic missiles — and last week he had the good fortune of support for this contention by Dr Henry Kissinger in a speech in Paris. But now, it seems, President Reagan has lost patience with the nightmare of nuclear strategy. He would make the discomfort go away by building a variety of novel defensive weapons based on Earth satellites. The impatience is understandable, but the way President Reagan plans to arrange that he sleeps easily at night is dangerous for the rest of us.

No security

First, if the President's ambitions could be realized technically, the new defences would stand in relation to modern strategic arms as did the never-built nuclear shelters of the 1960s to the old-fashioned strategic weapons of the times. For if it were possible for one of the superpowers to demonstrate that it could prevent the other's strategic weapons from reaching their targets, the other would quickly feel more vulnerable, so that the risk of a pre-emptive strike would be increased. One immediate consequence would be that the superpower feeling threatened would quickly build extra nuclear missiles, in the process abandoning the tactic and fortunate understanding of the past decade that the provisions of the unratified Salt II treaty will be observed. And then would come counter-measures, in this case satellites intended to put the other side's satellites out of action. In the long run, the effect would be to add another dimension to military technology. This only goes to show that lasting security cannot be secured by building new kinds of weapons while, as Dr Kissinger was complaining last week, a sense of temporary technical advantage is these days quickly eroded.

There are more urgent dangers. For the past months, President Reagan has been under pressure from his allies overseas to be more flexible in the two sets of arms control negotiations now under way at Geneva. The zero option for intermediate range missiles, the President's own proposal that all missiles within striking distance of Europe should be banished, is an idea that is probably unattainable. There remain other ways of delivering nuclear weapons on European targets that cannot be as easily counted and controlled — aircraft, British and French submarines, even howitzers. But settling on a negotiating position has been complicated not merely by the muddle about the appointment of a successor to Mr Eugene Rostow at the Arms Control and Disarmament Agency, and by the indiscretions of General George Rowney about the merits (or, in his view, the

opposite) of the members of his negotiating team at the Strategic Arms Reduction Talks (START), but by what seems to be Washington's inability to make a decision.

Ever since the German election on 5 March, the President has been reported to be on the brink of making up his mind. In the event, with last week's television broadcast, he has shown where his priorities lie. It is more important — this is how it will seem — to persuade Congress to back Mr Caspar Weinberger's ambitious budget than to get on with the arms control negotiations. This ordering of events will reinforce the widespread belief that the United States Administration is deeply suspicious of arms control in any form. There is a serious danger that the chances of success will be undermined.

And to what end? For several years, the Pentagon has been urging the military benefits to be won from new kinds of weapons systems, laser weapons and the like. Even so, it seems to have been the Heritage Foundation, the next best thing to the administration's ideological base, that has coined the winsome phrase "the high frontier" to sanitize the concept (see page 365). But are these novel weapons feasible? Fierce sceptics of the Pentagon's optimism abound, but those arguments cannot be settled without information that is not publicly available. One thing is, however, abundantly clear. Even if laser weapons to destroy the other fellow's warheads can with difficulty be constructed and made to function, like other physical systems, they cannot be 100 per cent effective. And they will themselves be vulnerable to attack. So the scientific community's response to the President's appeal for help should, in the first instance, be to remind him of these simple realities. The dream of an impenetrable shield against hostile nuclear warheads is an illusion. Strategically, however, a shield that leaks will ensure that future presidents will have to go on worrying about the strategic balance represented by missiles on the surface of the Earth and beneath the sea. This is not a recipe for sleeping easily at night.

Economics

It is also an expensive illusion, one that could well be literally ruinous. The Congress is sucking its teeth over Mr Weinberger's spending plans not so much because it objects to military strength but because even the present level of defence spending is largely responsible for the US government's huge budget deficit, which in turn keeps interest rates high and thus constrains the chance that the civil economy in the United States will quickly recover. Although some economists and many Administration spokesmen claim now to have seen the first swallow appear in the business statistics, economic recovery is still a delicate object and will take many years of steady growth for the United States and then the rest of the world to regain the sense of prosperity common a decade ago. But is not the hope that that will happen, and not be interrupted, the principal objective of the West's defences? And if the cost of that defence is itself so great as to put its chief objective at risk, is it not natural that people should ask whether the game is worth the candle?

The simple answer, of course, is that there are — or may be — better ways. The Administration's deep suspicion of the whole arms control process is itself belied by the remarkable way in which both parties to both Salt agreements, one ratified and one not, have stuck to the rules, and by the circumstance that there is



no known violation of any of the treaties signed in the past four decades. It is true, of course, that the temptation to cheat may increase as the stakes get higher, as they will be with treaties for reducing the numbers of strategic weapons on each side. But that circumstance argues for verification (where the scientific community to which the President was appealing could genuinely help). To take fright, to dither and confuse the negotiations under way by talk of novel weapons systems that are certain to damage only one thing — the economy — is to miss a trick and to hand the Soviet Union a powerful advantage. □

What now in France?

M. Chevènement was a powerful minister but his departure last week may bring stability.

THE departure of M. Jean-Pierre Chevènement from the ministry of industry and research in the French Government is the most spectacular but the most predictable of the consequences of the cabinet reshuffle. Since the Mitterrand government took office in May 1981, Chevènement's political position on the left of the Socialist Party has been well known and widely understood: he belongs to the school of thought that believes that a country such as France should be able to spend its way out of political trouble. At the outset, this opinion was shared by his fellow ministers. Only in the past few months, as the harsh realities of monetary arithmetic have become unavoidable, has the government come to recognize that it would have to change course and follow most other European governments in reducing public expenditure (or raising taxes). Chevènement now says that he offered his resignation on 2 February, eight weeks ago, when he saw the way the government was being forced to move and decided that he disliked what lay ahead. Plainly, however, the rift between him and the President was not so sharp as to prevent him keeping quiet so France could negotiate from strength (or lesser weakness) at last week's crucial meeting of the European Monetary System (from which Chevènement would have pulled out).

So what will happen now? Chevènement has been an energetic minister, responsible for science and technology until last August, afterwards for industry as well. On balance, he has served the scientific community well. Having persuaded the government that research and development are the only durable foundations of economic success, he fought successfully for budget increases whose like has become unfamiliar everywhere else. Last year, 1982, seemed like one long Christmas to many scientific institutions in France, but this year's generous budget increase has been sharply eroded by inflation and by the government's decision that a proportion of the funds appropriated should not for the time being be spent. But Chevènement has done more than merely provide more money. It is to be hoped that the spirits of the scientific community will permanently be raised by Chevènement's demonstration that at least one government is prepared to take it seriously, and to deal with it generously. So Chevènement will be missed, although not by everybody. The application of his syndicalist views to the management of scientific institutions, as in the proposal that institute directors should retire after a fixed spell in office, and his hankering for the electoral process in deciding who should be in charge (see p.366) may yet be disastrous.

So what will happen now? The French Government is unlikely radically to change its objectives and its attachment to the importance of research simply because M. Chevènement has gone. Its capacity to devote resources to these causes will, however, be diminished by the austerity measures that will be needed if France is to survive within the European Monetary System. M. Laurent Fabius, the new minister, is therefore likely to be as sympathetic as Chevènement. By reputation, however, he is less convinced than was Chevènement that innovation is valuable simply for its own sake. The result may be the best of both worlds. And it would be wrong to write off Chevènement, who remains a member of the National Assembly, and who is young and ambitious enough to keep pressure on the government. □

Down with air cartels

The US Justice Department is looking into airline price-rigging. Will it free this hamstrung technology?

CARTELS are widely and rightly distrusted in most industrialized countries — except when they are organized by the United Nations. That seems to be the tacit principle by which the explicit price-fixing carried out by the world's major airlines is condoned. At least once a year, the members of the International Air Transport Association gather at some delectable resort and then, without even a show of diffidence, make public announcements about the fares that they have agreed among themselves to charge on international routes. Very little imagination is needed to tell how the bargaining proceeds. The weaker international airlines, even when highly subsidized by their governments, cannot under present circumstances be driven out of business without putting at risk the bilateral agreements under which states offer each other landing rights on a reciprocal basis. Inevitably then, the financial needs of the weakest airlines are powerful determinants of the level of international fares. The stronger airlines, on the other hand, have no compelling reason to protest. They use some of the financial slack to experiment with discounted fares of various kinds or to keep down fares on domestic operations, while it must be presumed that they are also able to be less vigilant in the management of their own affairs than sheer efficiency would require of them.

These are some of the issues that should concern the United States Justice Department, now embarked on an important investigation of price-fixing on the North Atlantic route. Although the investigation appears to have been prompted by a legal complaint of collusive competition by the liquidators of the British company Laker Airways, which collapsed last year after failing to make a go of its cheap transatlantic fares, the Justice Department has apparently given itself wider terms of reference. So it should. The surviving airlines have been indecently quick to abandon cheap transatlantic air fares now that Laker has disappeared. And, as things are, the ultimate users of international air transport — fare-paying passengers — are probably paying more than they should for the benefits of an indispensable technology. So much should be clear from what has happened in the United States since domestic air transport was abruptly deregulated three years ago.

The same trick could not be made to work internationally, if only because government-subsidized airlines could always keep their aircraft decently full by undercutting their competitors, persuading their governments to pay part of the cost of other people's travel. Further diseconomies inevitably arise because bilateral agreements on international air transport usually limit the numbers of aircraft from the two sides that will be allowed to land each week at each other's airports, thus implying that, so long as fares are fixed, even the most efficient operators cannot hope to win a decisive advantage over their competitors. The way out of these dilemmas, however, is as clear now as it has been for several decades. The present system for regulating international air travel should be replaced by something quite different — a requirement that no international airline should subsidize its operations on any single international route coupled with the freedom to land where it likes as often as it pleases.

The consequence of such a system would be that airlines would be compelled to relate their fares to their true costs, even if by doing so they were to price themselves out of the market. (They might cushion the blow to their accounts by devising their timetables so as not to coincide with those of their competitors, as at present.) Only thus will over-capacity on the busiest international routes be made to disappear. Indeed, the advantages of confined competition along these lines would be immense, both for air travellers (who could expect to pay less) and the airlines (which would find themselves spending less on fruitless competition by shuttling half-empty aircraft about the world). While it has the chance, the Justice Department should take the issue by the scruff of the neck. □

US strategy

President Reagan opts for anti-missile defence

Washington

PRESIDENT Reagan's call last week for the development of technology that could "intercept and destroy strategic ballistic missiles before they reached our soil or that of our allies" has been received with taunts of "Star Wars" and "Buck Rogers" from Congress and expressions of scepticism and concern from many scientists. The chief concern is that space-based defence would trigger an arms race in space, endangering the surveillance and early warning satellites that provide a stabilizing force in the superpowers' nuclear stand-off.

The President has also provoked a certain ribaldry. Some have called him Ronald Ray Gun. The *New York Post* carried a headline "Star Wars to Zap Red Nukes".

Reagan acknowledged in his speech last week that this technology may be decades away. He ordered an intensive research and development programme as a top priority.

Congressional leaders questioned where the money was to come from and why it was not mentioned in the President's budget request.



Reagan specifically called upon the scientific community "who gave us nuclear weapons to turn their great talents to the cause of mankind and world peace: to give us the means of rendering these nuclear weapons impotent and obsolete". Thirteen scientists, including Hans Bethe, Frank Press, Burton Richter, Edward Teller, Harold Agnew, Charles Townes and Victor Weisskopf, were invited to the White House for a briefing on the proposal before Reagan's speech.

On the telephone, Hans Bethe expressed

several doubts about the proposal. "Suppose we got such a system and the Russians did not. Surely they would do all they could to frustrate that system". Bethe said he was "worried" that it could lead to "Star Wars" that would threaten the stabilizing role of observation satellites. Others, including Victor Weisskopf, have said that if either side were to deploy an effective defence, the other side would be left totally vulnerable to a nuclear attack and would feel compelled to knock out the defence — which could itself trigger a nuclear war.

Bethe also expressed doubts that a missile defence system could ever be practicable. "I cannot exclude that something like this could be workable, and it is better than previous proposals, but I have no idea how to do it. It would take many, many years" in any event, he said.

"If you want a defensive system that's useful against nuclear weapons, it has to be 90 per cent effective or better", he added. "It's not like anti-aircraft weapons in the last war. But many people in our present government don't see that difference."

Asked why he and the other scientists were invited to the White House briefing, Bethe replied, "That's the mystery. It's not clear why the idea was announced at this time, why at such an early stage of research." Bethe said the briefing was "very thin" and that the scientists "were allowed a couple of questions which were not answered very well".

The Soviet news agency Tass was quick to claim that the proposed system would violate the US-Soviet antiballistic-missile treaty. Caspar Weinberger, the US Secretary of Defense, conceded that the treaty might have to be amended if the system were ever deployed, but he defended the right of the United States to carry out research and development under the terms of the treaty.

The idea of space-based missile defence was articulated last year in a study by the Heritage Foundation, a conservative think-tank. The study, called the "High Frontier", directed by Lt General Daniel Graham, US Army (Ret.), called for the abandonment of the "bankrupt and basically immoral precepts of Mutual Assured Destruction". The study claimed that a first-generation "Global Ballistic Missile Defense" could be deployed in five to six years at a cost of \$10,000–15,000 million. This system would use self-propelled interceptors armed with conventional explosives and based on a series of satellites. The study also advocated increasing research on lasers and particle-beams, the second-generation systems, by \$100 million per year. **Stephen Budiansky**

Williamsburg summit

Agenda calls for collaboration

THE Western economic summit meeting arranged for Williamsburg, Virginia, in May can look forward to some relief from its usual sombre agenda of the problems of the world economy — an upbeat paper on the value of science and technology as an instrument of economic improvement.

The document, *Technology, Growth and Employment* (published in Britain as Cmnd 8818, HMSO, £3.55), has been prepared by a group of officials set up after the summit meeting at Versailles in June 1982, at least in part in response to President François Mitterrand's advocacy of the importance of technology. The chairman of the group was M. Jacques Attali, special counsellor of the President of France.

Two themes stand out in the report — the importance of basic research and the need for international collaboration. The report also includes a list of topics on which international collaboration is thought to have particular potential which reads a little like a delicate blend of projects governments have been unable successfully to manage on their own account and others they would like to get their teeth into.

Thus controlled thermonuclear fusion and photosynthesis are listed as deserving energy projects, while aquaculture, high-speed trains and biotechnology are all commended as fields in which collaboration could be fruitful. Conspicuous by its absence is most governments' present interest — information technology (but robots win a mention).

Some of the recommendations in the working group's report are unlikely, however, to be fully welcomed except as platitudes by the governments represented at Williamsburg — Canada, France, Italy, Japan, the United Kingdom, the United States, West Germany and the European Community. Thus the report urges that governments should support only basic research and long-term "high-risk" development projects, leaving more immediate projects to the private sector. The report also urges the importance of commercial competition as a means to the rational division of labour between states, asking in particular that governments should open their markets for public-sector purchases to all comers.

While much of the document is general (and familiar), each of the projects on which collaboration is commended is spelled out in such detail (together with the names of the countries that would be responsible) but in such modest terms that only the most stony summit will fail to agree with the proposals. The result should be a noticeable increase in the volume and scope of the funds available for international collaboration. □

Biotechnology

Genentech plumps for relaxin

Canberra

GENENTECH Inc. (San Francisco) will have exclusive rights to develop human relaxin, a protein sequenced after its gene was cloned in *Escherichia coli* by Dr Hugh Niall's group at the Howard Florey Institute, University of Melbourne, in collaboration with Dr John Shine of the Research School of Biological Sciences, Australian National University (see *Nature* 17 February, p.628-631). The agreement, signed by the Florey Institute and announced by the director of the institute, Professor Derek Denton, last Thursday, is believed to be Genentech's first foray in Australia. The company won the contract against British, Japanese and other American bidders by virtue of its track record and the attraction of collaboration with Genentech's scientists. Its terms ensure that the Florey Institute will accrue royalties, some of which will flow to the Australian National University, if the hormone is successfully marketed.

Relaxin is a peptide hormone synthesized in the corpora lutea of ovaries of pregnant women. It remodels the reproductive tract to facilitate the birth process by softening bone connective tissue to allow stretching during birth. But its therapeutic usefulness in alleviating the pain of childbirth or in conditions such as rheumatoid arthritis has yet to be demonstrated.

Corporate sponsorship of basic research is likely to increase in the virtual absence of government support specifically for biotechnology.

At present the dual support system for biotechnology funding — a A\$2.5 million allocation from the Australian Industrial Research and Development Incentives Scheme — proposed by the previous government is in abeyance. Since the foundations had already been laid by the Department of Science and Technology and the Australian Science and Technology Council, the new government accepted the system to avoid the delay and administrative duplication that a different scheme might entail. The Minister for Science and Technology, Mr Barry Jones, is enthusiastic about biotechnology but all government spending initiatives, including election promises, are being reviewed by the Minister of Finance, Mr John Dawkins, in the light of the A\$9.6 thousand million budget deficit for 1983-84 predicted by the Treasury.

Judging by what overseas biotechnology companies are spending on research and development, the proposed sum seems hardly adequate and unless implemented this financial year may not help scientists now busily signing contracts. Described by Niall as "a step in the right direction", the A\$5 million, as pointed out by Shine, will

not take a single product off the laboratory benches and carry it through the market.

In the meantime, Australian companies are playing safe. They will support only projects for which the financial returns seem assured. Most companies are prepared to take an idea only as far as necessary in order to patent it, and then sell it to a company that will take it the rest of the way. Only one company, Biotechnology Australia, which does in-house research and has the backing of Conzinc Riotinto Australia, appears capable of taking risks.

Vimala Sarma

Cool on malaria

GENENTECH, the California biotechnology company, has withdrawn from its negotiations with the New York University Medical Center (NYUMC) over the development of a possible vaccine against malaria. One issue that has arisen is whether a commercial partner can accept the terms set by the World Health Organization (WHO) in supporting the research.

WHO has long supported Dr Ruth Nussenzweig's malaria research at NYUMC, which has concentrated upon the sporozoite stage of the malaria parasite — the form injected into the bloodstream through a mosquito bite. In 1981, NYUMC decided that patents should be filed. The content of the patents has not been disclosed but they are presumed to cover the use of monoclonal antibodies, developed by Ruth Nussenzweig and her husband Victor against the sporozoite surface protein, to clone and express the gene for the protein. An account of this last step will appear in next week's *Nature*.

The initial stages of the project, which had not been carried out when Genentech first expressed interest in collaboration, have since been completed with the help of Dr Nigel Godson's laboratory at NYUMC. So far it is only the gene for a monkey malarial sporozoite that has been cloned.

Meanwhile the negotiations with Genentech were widely reported to have ground to a halt with its demand for exclusive rights on the vaccine, pitted against WHO's standard contractual demand for public access to any discovery emerging from the research it sponsors. Shirley Clayton, Genentech's treasurer, this week denied that that was a determining factor in the company's withdrawal from the negotiations. She said that negotiations were never more than very preliminary and have stopped because the company has decided to concentrate its efforts on projects already in hand rather than be distracted by new ones. Peter Newmark

Soviet space programme

International flavour

THE Soviet space programme is acquiring distinctive French and Ukrainian accents. Two major events last month — the launch of the Astron high-altitude satellite and the Comecon meeting in Kiev to prepare for the Halley's Comet mission — illustrate the French connection.

The French are to contribute equipment to the Vega mission (to Halley's Comet with a Venus fly-by) and also participated in the development of the ultraviolet telescope aboard Astron. Both events also had a strong Ukrainian slant — the 80 cm diameter optical telescope carried by Astron was developed by the Crimean Astrophysical Observatory, while the head of the special coordinating committee of Vega is Yaroslav Stepanovych Yatskiv, director of the astronomical observatory of the Ukrainian Academy of Sciences.

The choice of Dr Yatskiv — a mere corresponding member of the Ukrainian Academy — as head of so important a committee is surprising, since such posts are more than likely to be determined on the basis of rank. During the recent celebrations of the sixtieth anniversary of the Soviet Union, there was, it is true, a definite emphasis on the contribution of the academies of the fourteen non-Russian republics to All-Union and world science, whereas during the past two decades the usual trend has been to stress their role as a science base for local research and development. If, however, the chairmanship of the Vega committee was to go to a non-Russian, the most obvious candidate would at first glance appear to have been Academician Viktor Ambartsumyan, president of the Armenian Academy and himself a noted astrophysicist.

Moreover, although several of the Republic academies have established lead positions in certain fields (lasers in Byelorussia, pure mathematics in Lithuania, genetic engineering in Georgia), the speciality that immediately springs to mind for Ukraine is not astrophysics but cybernetics and also — as a result of the filial devotion of the academy's president Borys Yevhenovych Paton, who lauds it on every occasion — welding technology.

In fact, however, the Ukrainian observatories, in particular the Crimean and Khar'kov University observatories and the Principal Observatory of the academy, together with the academy's Institute of Radiophysics and Electronics, have already played a major role in the Soviet space programme. Techniques and instruments originally developed in Ukraine for meteorological observations of the oceans from Cosmos satellites were subsequently applied to mapping the surface of Venus. (Thus the planetological programme will

be continued by a descent module to be launched during the Venus fly-by by one of the two Venera probes, V-15 and V-16, used for the mission.) The Ukrainian interest in Venus is not confined only to the surface however — a Khar'kov team, using computer analysis or photographs taken through a red filter, has calculated the speed of rotation of the upper atmosphere of the planet to be of the order of 100 m s^{-1} .

Indeed, astronomical and astrophysical research in Ukraine ranges from Borys Ryabov's team in Khar'kov, which is studying thunderstorms on Jupiter using a 10-cm band radio telescope, to the new high-power radio telescope at the Crimean astrophysical observatory, and the relationship between solar activity and tree-ring growth (Kiev).

The Ukrainian Academy's Principal Astronomical Observatory moreover coordinates the work of Soviet observatories on the Earth's rotation and the determination

of time, and was the founder of the "USSR Planetary Patrol" sky-watch programme. In the Vega programme its special responsibility will be the ground-based back-up observations of the mission.

All this, however, does not quite explain why Dr Yatskiv, whose name does not even occur in the 1977 Soviet "Biographical Directory of Astronomers" (published in Kiev) should head the international co-ordinating committee for Vega. One not too serious suggestion, made by a fellow Ukrainian, was that the selection was made on linguistic grounds. There is no "H" sound in Russian, and hence the comet's discoverer becomes in Russian "Gallei". (Hence Ve-Ga for the joint Venus-Halley mission). Ukrainian, however, has an H (though no true G), so that Dr Yatskiv will be able to fulfil the prime requirement of an international coordinator — calling the object of investigation by its internationally recognized name.

Vera Rich

Czechoslovakia

Too many tasks, no responsibilities

CZECHOSLOVAK science is seriously hampered by government controls and the five-year planning system, according to a document of the Charter-77 movement that has now reached the West. This claim is not entirely surprising, for the Czech and Slovak media constantly harp on the shortfalls in what the planners see as the main "task" of science — serving the needs of the national economy.

Even so, in recent months, there have been signs of a growing disillusionment with all such official demands. Electronics, for example, is considered a key factor in current development plans, and as recently as the meeting of the Czechoslovak Scientific and Technical Society in February, the society's branch in the Prague Electrotechnical Faculty promised to train up to 3,600 specialists in electronics and microelectronics. Yet a few days ago Prague radio reported that factory managers are apathetic about sending employees to training and refresher courses in electronics.

The main interest of the Charter document (No. 26/1982) is the light it throws on the disillusion felt by the scientific community with the reiterated official line that science must be "present in every chapter of the plan" or, as party leader Gustav Husak told the Czechoslovak Academy of Sciences in February, "science is one of the crucial forces of social progress".

At the beginning of last year, the char-
tists began publishing a series of documents revealing inadequacies of the system that are glossed over or concealed by the government. This report, although signed by the three official spokespersons of the movement (Radim Palous, Anna Marvanova and Ladislav Lis) was clearly compiled by scientists who are struggling to function within the system.

Although some of their complaints (including shortage of funds, and in particular of convertible currency) seem to have an economic basis, the main difficulties have a clearly political background. Thus part-appointed administrators, ubiquitous at all levels of the scientific establishment, take key decisions on promotions, budgets and the permissibility or otherwise of foreign contracts. Publication of research results is strictly censored, and publication in foreign journals tacitly discouraged for fear of political repercussions.

The document also says that strict adherence to the five-year plan, and the needs of the economy, mean that radical new lines of research get little or no encouragement, initiative meets with no approval and informal working groups of scientists with common interests are virtually unknown. Weakest of all are professional contacts with foreign colleagues. All such contacts, even the most innocent and unsolicited routine correspondence (for example, to verify a reference in a published article), have to be reported to the authorities and fully accounted for on a special form. Such restrictions, the compilers point out, can only harm the ultimate development of Czechoslovak science.

Most serious of all, in the long run, is the virtually total neglect of basic research. In a covering letter, addressed to Prime Minister Strougal, the State Planning Commission, the Academy of Sciences and the Czech and Slovak Ministries of Education, the spokespersons add their own weight to this lack. Modern science, they point out, is largely "the practical consequence of theoretical thrusts of the human spirit (Descartes, Newton, Einstein *et al.*)". The present neglect of science for political motives is, they conclude, "tragic".

Vera Rich

UK pharmaceuticals

A matter of Life

LIFE, the national anti-abortion organization, has asked police in Birmingham to investigate a nearby abortion clinic which supplies the so-called "morning-after pill". Post-coital hormone treatment (PCHT) — usually a combined ethinyloestradiol/levonorgestrel formulation — is now widely prescribed in the National Health Service and in private clinics to prevent pregnancy up to 72 hours after unprotected intercourse. Life believes that PCHT is abortifacient and that anyone prescribing it is committing an offence under the 1861 Offences Against the Person Act. Interestingly, however, the organization has not made a complaint about the post-coital use of intra-uterine contraceptive devices (IUCDs), which is also widespread and is thought to work in a similar way in many cases.

Life's argument is that PCHT is not a contraceptive, because its mode of action is to prevent the newly fertilized ovum from implanting in the uterus. There has been no ruling in the courts on the legality of post-coital contraception, although guidelines published by the Department of Health and Social Security (DHSS) in 1979 do class the technique as contraceptive rather than abortifacient. DHSS takes the view that as "carriage" (that is, implantation) cannot occur in less than 72 hours following intercourse, there can be no possibility of procuring miscarriage before this time — which would indeed be illegal under the 1861 act if it was done recklessly or intentionally. However, the use of the word "carriage" in this sense is unknown to the editors of the Oxford English Dictionary, and Life argues (from its firmly declared position that legal rights begin at conception) that preventing implantation is tantamount to abortion — or as Life would probably put it, infanticide. There is no suggestion that the clinic in question has used PCHT after the 72-hour limit.

If it is established that PCHT is abortifacient, its use would have to be justified on medical grounds by two doctors in order to comply with the 1967 Abortion Act. However, even in this case a successful prosecution would appear unlikely, since the 1861 Act would require proof of *intention* to procure miscarriage. The defendants would probably be able to argue, after taking the best advice available, that no such intention was present.

IUCDs are also thought to act by preventing implantation, whether used pre- or post-coitally. Three years ago the Director of Public Prosecutions declined to prosecute Professor Peter Huntingford of St Mary's Hospital Medical School for inserting an IUCD as a pre-coital contraceptive, when he attempted to start a test case. But Life appears unwilling to take on the estimated 250,000 IUCD users in Britain.

Tim Beardsley

French science administration

Chevènement eclipsed

Paris

JEAN-PIERRE Chevènement, French minister of state for research and industry, political motor of the new French technological revolution, has gone, eliminated in the drastic pruning of the French Government last week which reduced the number of cabinet ministers from 34 to 14.

Hitherto, Chevènement has encountered few setbacks. He mobilized France for science, and saw through a law for research meant to guarantee a 17.8 per cent annual real growth rate in French civil research spending to 1985. He took control of all the major research organizations and established effective means of cooperation between universities and industry.

Chevènement's reverses were chiefly the result of characteristic haste, and the opposition this generated.

He followed through his claim that "without a political strategy of research, there can be no political strategy of industry, society or culture" when he became minister of research and industry in June last year but was not at ease in his new role. Private industry claimed he neglected it, while he complained at the inertia of the nationalized sector. This led to an emphasis on rather premature decisions to the neglect of concrete action, and he was accused of excessive intervention.

At the same time, the research community felt abandoned. Although, under pressure from Chevènement, science was exempted from last year's 25 per cent freeze on public spending, leaving a net real increase of 10 per cent over 1981, the Prime Minister's policy of "rigour" advertised for 1983 was a greater menace. Despite the promises of the "loi", the net increase this year will be zero if the rigour applies to research.

Important though these problems may be, however, it is not clear that they are sufficient to explain the resignation of a minister with a reputation for courage. Many point to a political divergence between the leader of Ceres, the quasi-Marxist left wing of the socialist party, and the Mauroy--Delors group. Besides, Chevènement's difficulties in the recent

local elections (he was reelected at Belfort, but only on the second vote) will not have increased confidence in him.

On 22 March, the day his departure was announced, the ex-minister of research and industry issued a communique saying he had offered his resignation on 2 February, because of his disagreement with "the method and conception of the actions of the government". Chevènement has been a fierce opponent of the policy of austerity adopted by the finance minister, M. Jacques Delors, who has now become the strong man of the new government.

Even so, many believe that by quitting now, Chevènement is leaving open the possibility that in the long run M.

Mitterrand may call him back. He would then return untainted by the compromises of "rigour".

What now of research? Already the name of the ministry has changed: no longer research and industry, but industry and research. The new minister is M. Laurent Fabius, ex-minister of the budget, a man close to M. Pierre Mauroy, the Prime Minister, and certainly more at home with financial realities than with great issues of industrial regeneration.

Isabelle Trocheris

Robert Walgate adds:

M. CHEVÈNEMENT never seemed to be a man who would compromise: he came on stage with a dream, enthused many, and left in the same mood. But his supreme confidence in his own concept of science for France, in his scheme for regenerating French industry within a fortress of trade barriers, began to seem like arrogance.

CNRS elections

Colleges indulge democracy

Paris

By an irony of timing, on the day before the French Prime Minister, M. Pierre Mauroy, announced his new government from which Jean-Pierre Chevènement, minister of research and industry, was dropped, the Centre National de la Recherche Scientifique (CNRS) passed a decisive landmark in the new organization decreed by Chevènement with the election of the sections of its national committee.

As a consequence of Chevènement's famous "loi" (law), adopted by the Assembly last summer, and of the reorganization of CNRS flowing from it, the new "parliament" of CNRS, the consultative committee of researchers in which various fields of research are separately represented, has been enlarged (from 41 to 45 sections) while active researchers are more heavily represented.

Under the new arrangement, 16 members of each section are elected by ballot and electors can vote either for independent candidates or for candidates on lists proposed by the unions — one of Chevènement's innovations.

Last week, there were 720 places to be filled in three separate electoral colleges. The results of the election are as follows:

● **College A** (270 places). This category, which includes directors of research, members of academies and professors at the Collège de France, inevitably offered the greatest proportion of independent candidates. In the event, 139 individuals were elected together with 25 members of moderate or right-wing unions and 106 members of unions closer to the socialists.

● **College B** (270 places). This category includes all CNRS researchers together with researchers of various kinds, at universities for example. The results of the election are more favourable to the government, with 244 seats going to left-wing unions, six to right-wing unions and only 11 to independent candidates.

● **College C** (180 places). This category includes engineers, technicians and administrators. Of the seats now filled, 144 have gone to left-wing unions, 33 to more moderate groups and only three were independent candidates. At this year's election, there was a much better turn-out than in 1978 — 70 per cent overall, with 80 per cent in College A.

In spite of these encouraging results, there was some disquiet at CNRS when Chevènement's departure was announced, principally because the 16 elected representatives of each section will now be supplemented by nine suitably qualified people nominated by the new minister on the recommendation of the director general of CNRS.

The arrival of the new minister, M. Laurent Fabius, could hold up the nomination and, as a result, delay the functioning of the national committee, which has been in suspension during the long months preceding its reform. At the beginning of March, M. Chevènement was promising that the hiatus in the affairs of CNRS would be ended before the month was out.

Isabelle Trocheris



Chevènement left — Fabius right

University of London

Merger, merger everywhere

THE saga of the restructuring of teaching at the University of London, approved by the senate of the university in April 1982, is unfolding slowly. Shortage of funds for building projects is hampering progress towards some of the mergers that have already been negotiated and is delaying others yet to be finalized.

The reorganization was made necessary by a sharp drop in the budget of the university as a whole that threatened some of the smaller colleges with severe reductions in the numbers of courses they could offer. The University Grants Committee has, since the cutbacks were announced in 1981, relented a little and has allowed the targeted reductions in student numbers to be

finish up with surplus real estate on its books. St George's Hospital, now relocated in south London at Tooting, has surplus space originally built in the expectation that Chelsea College would move there. Now there is pressure to concentrate the teaching of pharmacy on that site, which would imply that the School of Pharmacy in Bloomsbury could be vacated. Bedford College is transferring its science teaching to Royal Holloway College, which has a 100-acre "green-fields" site at Egham, 20 miles to the west of London. One anxiety is that this site will be less attractive to would-be students.

Bedford was in need of new premises because leases on the existing site in



Counter-attraction. Bedford's Regent's Park site has assets unmatched by Egham

delayed by a year — a concession extended to only a few other universities.

The consequences for science teaching will eventually be profound. The aim is to concentrate on teaching at five major sites. So much emerged from the work of a number of university committees which last year painted with a broad brush the future pattern of teaching in broad subject areas, and which recommended a concentration of teaching on five sites, not always the same. The principle was, however, accepted by the university's joint planning committee and eventually by the university's senate, which nevertheless shrank from saying which they should be for lack of the power to dictate to colleges which are, in principle, autonomous.

Whatever the outcome of the organization now under way, the University of London is almost certain to

Regent's Park are due to expire early next century. Bedford has to raise £16 million to effect the move, and has applied to the University Grants Committee for the extra funds likely to be needed if the move is to be completed by the target date of the end of the 1985-86 academic year.

Kings College, based in the Strand, had earlier contemplated a merger with Bedford but has now agreed to merge instead with Queen Elizabeth College (QEC) in Kensington, which is exclusively science-based. Chelsea College, which was, with QEC, one of the most seriously threatened colleges, and which is also predominantly science-based, is now also negotiating and "actively planning" for a joint college with QEC and Kings, to have 6,000 students, as large as any other in the University of London. If this merger goes through, the combined college, which will

include a medical school and an engineering faculty, will have a student body with two-thirds science students, compared with one-third at Kings at present.

The new college will, however, have to occupy a reduced number of sites. Which of the sites used by the three colleges will be maintained has not yet been decided, but it is assumed that the Strand site will not be abandoned. The long-term objective is to concentrate on two sites, and QEC's Kensington site seems the most vulnerable. But it has been agreed that the site will be used for "the foreseeable future". Professor H.J.V. Tyrrell, vice-principal of Chelsea College, says that staff reductions entailed by the move, although regrettable, would have occurred anyway if the college had attempted to "go it alone".

Negotiations are also under way for an "association" between Westfield College in Hampstead and Queen Mary College in east London. The vice-chancellor of the university, Professor Randolph Quirk, is keen to see more science being taught at the east London site, in an area where substantial industrial growth is expected and links with industry might profitably be forged.

If the plans do go ahead, it is expected that science teaching at Westfield College will be moved to Queen Mary College and arts subjects at Queen Mary College will be moved in a reciprocal transfer to Westfield's Hampstead site. Imperial College and University College — two of the largest colleges in the university — will continue at their existing sites.

Tim Beardsley

Letting off steam

THE greatest use of energy in the United Kingdom is boiling water to make steam — yet there has been no study of the UK boiler stock since 1953. Until, that is, last week, when two energy specialists from the Science Policy Research Unit of the University of Sussex published a review. The potential implications are important: 63 per cent of industrial fossil fuel in Britain meets its end in boilers, say the researchers, and boilers could, in principle, burn anything. So a country's energy politics could be determined by its boilers.

In particular, some argue that coal could substitute for oil in this sector. However, only 3 per cent of the boilers installed in Britain in the past 20 years were coal-fired, says the report. Coal is cheaper to burn than oil, but coal-fired boilers cost twice as much as oil-fired ones (because of the ash). Replacement of oil-fired boilers, even at the end of their life, by coal-fired ones is at present uneconomic, even allowing for a 25 per cent government grant. The pay-back time is four years; industry normally demands two years. The picture is unlikely to change for another 20 years, the researchers say, until the oil-coal price ratio rises higher.

Robert Walgate

US Environmental Protection Agency

Political pollution unabated

Washington

CONGRESSIONAL investigators last week released documents suggesting that a White House official had sought to manipulate the awarding of hazardous-waste clean-up grants in order to help selected candidates in last November's elections. The documents add credence to earlier testimony by Environmental Protection Agency (EPA) officials before the investigations subcommittee of the House Energy and Commerce Committee that they had been ordered to speed up work on certain grants so that they could be announced before the election.

The new evidence also, for the first time, implicates the White House directly in the growing scandal at EPA. According to calendars turned over to the investigators, Rita Lavelle — the EPA official in charge of hazardous waste — met James Medas, a special assistant to the President for inter-governmental affairs, on 13 July last year. Notes taken at that meeting by Lavelle's assistant, Susan Baldyga, show that the discussion was devoted to a rundown of state governors' election campaign "races".

Baldyga's notes, headed "Jim Medas/go through the races", includes comments: "New Jersey — Keene (*sic*) — help him all we can" and "New England/Bend over backwards/Snelling/Edward King". Thomas Kean, the Republican governor of New Jersey, was reelected in a very close race against Democrat James Florio, a member of Congress. Richard Snelling is the Republican governor of Vermont, also reelected; Edward King, conservative Democrat, lost in his bid for reelection as governor of Massachusetts.

More revealing are several pages of notes found in Baldyga's office. These list candidates state-by-state and also contain clear references to hazardous waste sites. The notations "no political hay" and "no new sites" appear frequently on the list, as do indications of candidates selected to make "announcements". Another of Baldyga's notes, referring to the Tar Creek waste sites in Oklahoma (which EPA had listed as one of the 418 priority sites), bluntly states, "Status on Tar Creek/Political way to play Tar Creek". EPA awarded \$435,000 on 1 July for cleaning up Tar Creek.

A check on hazardous-waste sites for which clean-up funds were authorized in the three months preceding the election shows that the largest number in any one state — 17 — was in New Jersey. New York was next with 8. Both Lavelle and Baldyga were fired from EPA during the first shake-up at the agency last month.

Medas, in a statement released by the White House, said "At no time did I say, suggest or imply that EPA policies ought to be shaped to meet political considerations". The White House had earlier said that Lavelle and Medas discuss-

ed only "political contacts they had in California". After Baldyga's notes were released, Medas admitted discussing some races but not waste sites, with Lavelle.

Meanwhile, in an effort to get the beleaguered agency swiftly back in action, President Reagan last week announced that William Ruckelshaus, the first EPA administrator, would replace Anne Burford as head of EPA. Ruckelshaus, who served under President Nixon in the early 1970s, was considered an environmental moderate. He was also acting director of the Federal Bureau of Investigation under Nixon and deputy attorney general until he resigned in 1973 in the "Saturday night massacre" when he refused to carry out President Nixon's orders to fire Watergate special prosecutor Archibald Cox.

Late last week, there were more shake-ups at the agency. John Hernandez, the deputy EPA administrator who has served as acting administrator since the resignation of Mrs Burford, was asked to resign by President Reagan. Hernandez has himself been at the centre of controversy for allowing Dow Chemical Company to rewrite a report on dioxin contamination around a Dow plant. Also asked to resign were Robert Perry, EPA's general counsel, and John Todhunter, assistant administrator for pesticides and toxic substances. White House officials said this was to give Ruckelshaus a free hand in bringing in his own staff.

Stephen Budiansky

Third World science

New centre

Bangalore

SCIENCE and technology have an important role to play in promoting socio-economic growth in developing countries. That was one of the points made at the seventh Non-Aligned Summit (NAM) Conference of 101 Third World countries held recently in New Delhi. On the very first day, it was decided to create a centre for science and technology for non-aligned countries with its headquarters in New Delhi.

In her inaugural address to the conference, Mrs Indira Gandhi, the Indian Prime Minister, called for cooperation among the developing countries in the spheres of "agriculture, irrigation, plant genetics, public health, technical training and small industries". Pointing out the role of science in eradicating poverty, Mrs Gandhi observed that "at present 90 per cent of the world's research is not relevant to us because it is earmarked for the priorities of the technological leaders. Each developing country must therefore strengthen its domestic base in science and technology, and we should collectively devise more effective mechanisms for the pooling of our experience". She added: "Some people still consider concern for the environment an expensive and perhaps unnecessary luxury. But the preservation of the environment is an economic consideration since it is closely related to the depletion, restoration and increase of resources."

Radhakrishna Rao



The 1981 world league table of nuclear power users in which countries are ranked according to the percentage of their electricity generated using nuclear power has been released by the International Atomic Energy Agency (IAEA). IAEA puts France as the top producer and Finland in second place. However, provisional figures for 1982 show this situation to be reversed, with Finland generating 40.3 per cent of its electricity through nuclear power and France generating 48.7 per cent.

Future for magnetic fusion

SIR — On 22 February 1983 the General Accounting Office (GAO) released a preliminary report that I had requested last year on the status of the Department of Energys (DoE)'s implementation of the Magnetic Fusion Engineering Act of 1980.

The request for the study was made in January 1982 in response to the Reagan Administration's proposed budget cuts in the US magnetic fusion programme, and the subsequent resignation of Dr Edwin Kintner, associate director of the Office of Fusion Energy at the Department of Energy, in protest at these cuts. I felt strongly at that time that the Administration's cuts were undercutting and circumventing the intent of the Magnetic Fusion Engineering Act, and subsequently Congress's ability to make choices that would allow the goals of the act to be met. By so severely restricting the funding for research into magnetic fusion energy, DoE was restricting and possibly eliminating its ability to follow through with the provisions of the act that had received such overwhelming support in 1980.

The report by GAO found that DoE was changing its plans to follow through with the provisions of the Magnetic Fusion Engineering Act, and had not informed Congress, the architects of the act, of these decisions. GAO states, "Citing budgetary constraints, DoE no longer plans to follow through the act's intended development strategy", and yet Congress has received no notice of such action, and thus continues to assume that the provisions of the act are being adhered to in DoE planning.

In further examining the intent of the act, GAO states: "In addition to changing development strategy, experts, including the National Academy of Science's National Research Council, are also questioning whether DoE is adequately pursuing alternative concepts so that options are available when the decision is made to construct the next level reactor". By further abandoning the development provisions of the Magnetic Fusion Engineering Act, and limiting research into alternative concepts of magnetic fusion (that is, research not a part of the lead tokamak project at Princeton University) the United States will have little choice when the time comes to pursue the next phase set out in the act.

The act foresaw the advancement of several magnetic fusion concepts such as mirrors, stellerators and bumpy toruses, in addition to tokamaks, so that an informed decision could be made about which concept to pursue toward commercial viability. Mirrors, the most advanced of the alternative concepts, may not even be ready for comparison when that time comes. GAO states:

The Mirror Fusion Test Facility (MFTF) is presently under construction. It is a larger facility than the Tandem Mirror Experiment,

comparable in size to the Tokamak Fusion Test Reactor. Scientists also hope to demonstrate scientific breakeven on the Mirror Fusion Test Facility before DoE makes a decision on the confinement concept to be used in the next level fusion reactor. DoE expects to make this decision in 1987. However, since funding reductions have delayed construction of the MFTF until at least 1985, it appears unlikely that scientific comparability between tokamaks and mirrors will be achieved by 1987.

Thus the possibility of a knowledgeable and informed choice based on adequate scientific information, as specifically required in the Magnetic Fusion Engineering Act, is impossible. But again, Congress has not been advised of this policy change.

The result of current policy is that due to funding reductions insisted upon by the current Administration, we are faced with a programme whose directions and goals are being changed by bureaucrats forced to function within these budgetary constraints. Congress is being kept uninformed regardless of current law that specifically requires such information at frequent intervals. And we have a programme whose purpose is being abandoned in the name of cost savings, with no eye to its future benefits.

It is clear from the GAO report that DoE is disregarding the crucial legislation that Congress intended to be the framework for the national magnetic fusion effort. If this nation is ever to reach the goals of clean, safe, unlimited fusion energy, we must refocus our efforts and follow through with the Magnetic Fusion Engineering Act.

FORTNEY H. STARK

House of Representatives,
Washington, DC, USA

Where intelligence tests fail

SIR — Recent attempts to adjust the mean IQ scores of Japanese and American samples (*Nature* 24 February, p.655) may have left unstated the lack of theoretical resolution that attends inter-generation or cross-cultural test score comparisons. All psychological inference from performance demands firm construct validity. Nevertheless, the legacy of the testing paradigm is the need for an agreed structure of intellect. Since Spearman's general factory theory, one revision every decade has emerged: and the names of Thurstone, Burt, Thomson, Guildford, Cattell, Carroll and Sternberg are linked with various notable attempts. Their efforts testify to IQ score complexity but admit no certainty.

Given disagreement in the parent culture, transposition of a test to a host culture requires proof of psychological equivalence before means are compared. For more than forty years cross-cultural

psychologists have pursued a set of procedures to establish equivalence. A great variety of approaches have been tried. Specialists in this area would recognize the contributions of Biesheuvel, Coffman, Mellenbergh, Poortinga, Schoeneman, van der Flier and Vester. Their work too is still far from complete. Consequently, psychologists who venture across cultures or generations with test scores as dependent variables face scientific hazards. Double jeopardy awaits those who risk cross-cultural and inter-generational comparisons at one and the same time.

The problems of equivalence within generations may be understood thoroughly; but within-culture-across-generation issues have not had close conceptual, let alone empirical analysis. The most tenuous comparison of all, across generations-across cultures, points in the direction of a *reductio ad absurdum*. The general practice of inference from group averages requires scientific credibility; and this, at the moment, cannot be guaranteed¹.

S.H. IRVINE

Plymouth Polytechnic, UK

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Power corrupted

SIR — In the recent note referring to Hooke's *Micrographia* (*Nature* 3 March, p.9) it was stated that this "was the first book in English to be devoted to microscopy". This is not so. This magnificent work has many claims to fame but being the first in its field in English is not one of them. Published in 1665, it was anticipated by Henry Power's *Experimental Philosophy*, which appeared the previous year and of which the microscopical part had been prepared some years earlier. This, as part of its extensive title informs us, was written *In Three Books: Containing New Experiments, Microscopical, Mercurial, Magnetical*. Thus, like Hooke's *Micrographia*, its contents are not confined to microscopy.

Power's microscopical observations cover a wide range of objects, both animate and inanimate, and include some truly original work on biological materials. Clearly, concisely and entertainingly written, the book suffers from the lack of that feature which makes the *Micrographia* so attractive — its splendid plates — for it contains no more than a few crude sketches by way of illustrations: nor can its text be said to be the equal of Hooke's. Nevertheless, for its time it was a work of considerable merit, and among its distinctions is that of being the first book in English to be devoted to microscopy.

GEOFFREY FRYER

Freshwater Biological Association,
Ambleside, Cumbria, UK

Final words on malaria's return

SIR — The evidence offered by Sharma and Mehrotra¹ does not change our major contentions².

The introduction of high yielding crop varieties has led to the resurgence of malaria in much of southern India's agricultural zone, not because of any inherent feature of the new seeds but because of the technology commonly employed in growing them. Specifically, the intensive use of chemical pesticides leads to increased *Anopheles* resistance to these chemicals, and to resurgent malaria.

On the question of whether insect susceptibility to chemicals can be reversed, it is possible that mutation may be one of the mechanisms by which individual mosquitoes become resistant to a particular chemical. However, a far more widespread cause of resistance is that of genetic selection within the insect population at large. This phenomenon does not appear to be reversible. In fact, the genetics of resistance suggest that an apparent return to susceptibility may actually be only short-lived. According to Robert Metcalf, for example, "although the gene frequency of a specific resistant allele may decrease upon the removal of insecticide pressure, there persists a changed background of residual inheritance in the genome that causes the strain to regain its resistance as soon as the insecticide is reapplied. . . These factors prevent the successful long term re-use of any insecticide in insect populations with resistant alleles, even though the initial resistance has apparently reverted to full susceptibility." (ref.3). It is thus clear that both farmers and malaria control specialists will have to search for new methods to control their pests.

This brings us to the problem of producing enough food for a growing population. Clearly, developing countries cannot afford to decrease their agricultural yields. By calling for a reduction in pesticide use, we do not mean to imply that food production is less important than malaria control. Rather, integrated pest management offers a potential way out of this impasse and has been successfully implemented with high yielding varieties of cotton, corn, sugar cane and rice in many areas of the developing world, including India^{4,5}. With carefully designed integrated pest management systems, countries can continue to increase the production of basic foodstuffs while reducing the selection pressure on *Anopheles* mosquitoes and other insect vectors of disease.

GEORGANNE CHAPIN
ROBERT WASSERSTROM

Columbia University,
New York, USA

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SIR — To settle the controversy raised by Chapin and Wasserstrom¹, we further substantiate that:

Nearly two decades of DDT spraying in human dwellings and cattlesheds did not induce any resistance in exophilic vectors of malaria such as *Anopheles philippinensis*, which also breeds in paddy fields². Similarly, *Anopheles balabacensis* has remained susceptible to DDT in India and Bangladesh, although intensive agriculture is being practised in areas of its distribution³. *Anopheles sudaicus*, the vector of malaria in Andaman & Nicobar island, has remained susceptible, even though liberal use of pesticides has been made to increase agricultural production⁴. The inability of exophilic vectors, in contrast to endophilic vectors, to develop resistance, was attributed to the mosquitoes' reluctance to rest indoors on DDT deposits.

Chemical pesticides used in India to increase agricultural production of rice and cotton did not induce any resistance in the primary target species of these crops⁵ as happened elsewhere⁶.

One of the most difficult obstacles to the successful control of insect pests is their ability to develop resistance, which is pre-adaptive. Some countermeasures are being investigated but none is at present available for large scale operational use, except the replacement insecticides⁷. Pest control must thus be managed more judiciously until newer technology becomes available for national programmes.

Under the modified plan of operation of the National Malaria Eradication Programme of India, malaria control is based on indoor residual spraying of insecticides, and free drug distribution in even the remotest areas of the country. The programme costs an annual Rs.1,000 million and there are persistent demands for increased allocations. In India the basic reproduction rate of malaria, in large parts of the country, is very high. With this background, a relaxed view in favour of integrated pest management may be highly detrimental and result in untold human suffering.

V.P. SHARMA

Malaria Research Centre (ICMR),
Delhi, India

K.N. MEHROTRA

Indian Agricultural Research Institute,
New Delhi, India

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This correspondence is now closed —
Editor, *Nature*.

Rescue mission for British mussel

SIR — I was most interested by Dr R. Robert's review¹ of Isom and Hudson's recent report about a culture technique for the glochidia of freshwater mussels². The British species, *Margaritifera margaritifera* (L.) is indeed endangered and in need of urgent conservation measures, however, we unfortunately feel that Isom and Hudson's technique will not be of great relevance in Britain, at least at present.

It is already technically easy, although rather laborious, to infect stock fish with glochidia and then retrieve viable mussels from them. The real difficulty which must be overcome before *M. margaritifera* can be restocked successfully, is that no-one has succeeded in rearing the young mussels after their release from their host fish. They are tiny (0.5 mm diameter) and have proved impossible to rear for more than a few months. All attempts at introducing such young mussels into even very favourable natural sites have so far failed and until this problem can be overcome glochidial culture will not help.

Our view³ is that urgent conservation measures must be applied to restrict over-fishing. In Scotland almost all populations have been fished for pearls, to the point where their survival is in doubt, and unless the mussel is to be reduced to a few, isolated populations in protected areas, action is needed. What seems most unlikely is that the political and economic climate will allow measures which will work and which can be effectively policed.

MARK R. YOUNG

JENNIFER C. WILLIAMS

Department of Zoology
University of Aberdeen, UK

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Crno!p pltohg

SIR — I have long regarded your journal as a valuable source of news about the world of science. However, if I judge the accuracy of the general content by a few items of which I have direct personal knowledge, I must begin to question its reliability. I refer to three examples in the issue of 27 January: One of the Great Lakes has become Eyrie instead of Erie (p.285); the director of Fermilab has been changed from Leon Lederman to Leon Letterman (p.273); and Big Brother, created by George Orwell, has been ascribed to Aldous Huxley (p.271). If you fail in these small things, how can we trust you in the large?

GEORGE L. TRIGG

(Editor, *Physical Review Letters*)
New York, USA

A visit to the bombed nuclear reactor at Tuwaitha, Iraq

In June 1981, aircraft of the Israeli Air Force bombed the Iraqi research reactor 25 km from Baghdad and put it out of action. Richard Wilson, head of the Physics Department at Harvard University, describes a recent visit.

IRAQ has a nuclear research centre at Tuwaitha, some 25 kilometres south-southeast of Baghdad which was set up with the help of the Soviet Union. Iraq was among the first countries to sign and ratify the nuclear Non-Proliferation Treaty (NPT) by which they undertake not to build or acquire nuclear weapons, and keep open their facilities to the international inspection requested by the rest of the world.

By signing NPT Iraq gave up some of its sovereignty, but the Iraqis did not advertise that they were in favour of peace, and many people thought they were making bombs. In March 1982 at the Second Arab Energy Conference at Doha, Qatar, I talked to several Iraqis, including the oil minister, Mr Tayeh. I emphasized that by allowing this ambiguity to persist they were getting the worst of all possible reactions from the rest of the world. I urged that they invite foreign visitors. Last December I received an invitation to visit from the Ministry of Foreign Affairs.

I visited the centre from 27 December 1982 to 1 January 1983 and was shown around by the director, Dr Abdul Kardr Ahmed. I was encouraged to ask questions and to take photographs of all scientific in-

stallations and of people with one exception; they did not want cameras inside the Soviet reactor building.

The research centre has been operating for many years. It shows obvious signs of being influenced by the Soviet Union. The centre has two parts (see Fig. 1): in the middle lies the old part, with a pleasant garden-type atmosphere; and outside are the new facilities under construction with a bare construction site appearance.

Since the Tammuz 2 reactor was bombed in June 1981, an earth berm some 100 feet high with anti-aircraft guns and radar on top has been built all around the site in a roughly square arrangement a quarter of mile on a side. To the east of this a main road (the road from Baghdad to Kut and the easterly route to Basra) has been diverted and further 100-foot mounds with anti-aircraft guns on top can be seen for 2 miles around. I was not allowed to photograph these military installations.

The old 10 MW Soviet reactor (WWR-c) is in a typical Soviet installation with no containment vessel. The building, floor tiles and reactor are copies of those at the Institute of Theoretical and Experimental Physics (ITEP) in Moscow or at the In-

stitute of Nuclear Physics, Garchina, near Leningrad, or the 2.5 MW research reactor at Inchass, near Cairo, Egypt. The reactor is listed in the International Atomic Energy Agency (IAEA) 1974 list as a 2 MW reactor and started operation in 1968, but it has since been upgraded (it is listed in a 1976 US Department of Energy report at 5 MW).

The procedures are also as used in the Soviet Union. At British, American and French research reactors, people enter the containment vessel through an airlock but do not change shoes; in Soviet reactors, and at Tuwaitha, the authorities insist that paper overshoes are worn in order to control the possible spread of radioactivity.

The reactor is light water moderated, swimming pool type fuelled with uranium 90% enriched in uranium-235. Although a full fuel load for the reactor is about 4 kg of uranium, I was told that 12 kg of uranium fuel is regularly kept on site. It is of course subject to IAEA safeguards.

The reactor is above ground. The reactor core centre is about 3 feet above the floor with neutron beam ports at that height. The neutrons are at present used for the following experiments:

(1) Neutron capture γ ray detection and measurement, using a germanium (Ge(Li)) detector bought from ORTEC, Oak Ridge, Tennessee in the United States.

(2) Inelastic scattering of reactor fast neutrons. The γ rays are detected in two germanium detectors (also ORTEC) and the neutrons in a set of about eight scintillators 8 feet long looked at by photomultipliers at each end. The neutron energy is measured by time of flight. Standard circuits from Lecroy (US) are used, connecting via a CAMAC (European) crate to a Hewlett Packard computer. This work was begun as a collaboration with scientists from the Kurchatov Institute of Moscow, solely as a study of the γ rays from inelastic neutron scattering. A big compendium of this work was published (text in both Russian and English) by Atomizdat of Moscow in 1979. They use separated isotopes obtained until now from the Soviet Union.

(3) Two γ -ray detectors for neutron capture γ rays, one detector of sodium iodide (NaI) and one of germanium (Ge(Li)).

(4) A double axis neutron diffraction spectrometer, computer controlled.

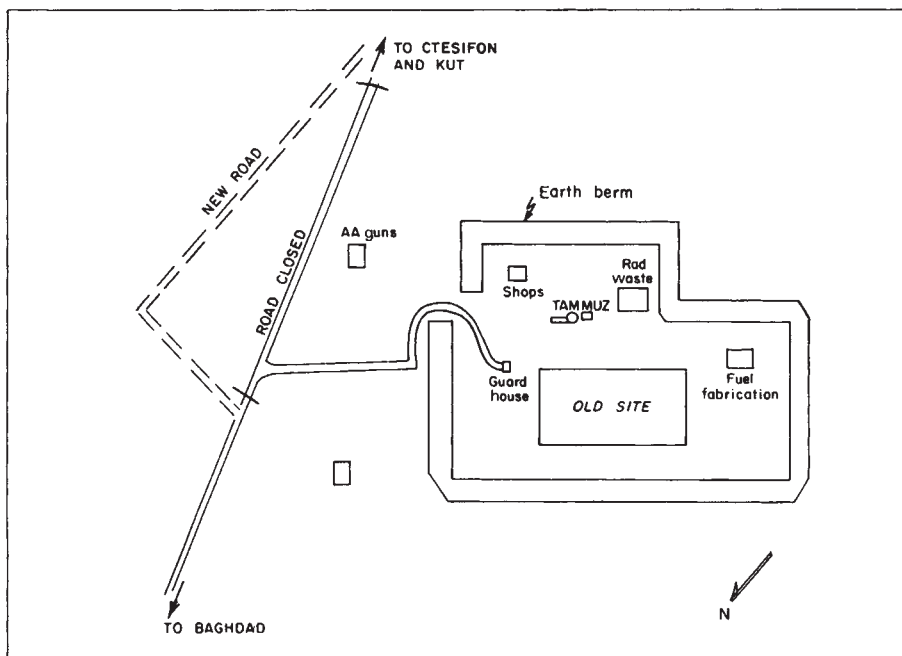


Fig. 1 Sketch of Iraqi nuclear research centre.

(5) A rabbit tube for short lifetime isotope production and activation analysis.

The leading physicist at Tuwaitha seems to be Dr Ja'afar Dhia Ja'afar, who has a PhD in physics (experimental high energy) from Imperial College, London. Ja'afar is certainly well respected both inside and outside Iraq; he talks well and until 3 years ago travelled to the European Centre for Nuclear Research (CERN) in Grenoble, and other places in the West. It will be remembered that Amnesty International and others were concerned about Ja'afar's disappearance from society in January 1980. This was said to be due to his complaining officially about the arrest in October 1979 and trial in March 1980 of Dr Husain Al Shahrastani, who was head of the chemical engineering group at Tuwaitha. Although it was reported to Amnesty International that Dr Shahrastani was executed in April 1980, Dr Ghofar, vice chairman of the Iraqi Atomic Energy Commission, stated that he is serving a 6-year jail sentence. The alleged crime was sedition; Shahrastani is a Shiite Moslem and had been visiting his Ayatollah, Muhammad al-Bakr al Sadr, who has since been executed. It was reported that Ja'afar was sent to a sanatorium, but he is now free, and Dr Ghofar reports he had had a "vacation".

There is a large section at the centre studying nuclear applications to agriculture and biology under the leadership of Dr G. R. al Khabeab. Most of this work is beyond my understanding. But I understood the following uses of radioactive sources: (1) Soil physics — use of tracers to study the migration of radionuclides (transport of radionuclides in soil), and (2) the use of cobalt-60 irradiation to cause mutations and hopefully better plants (better meaning high yield, resistance to disease). (The cobalt-60, I was told, was bought from large reactors in other countries since the small reactor is unsuited to its production.)

The new reactor, Osirak, that was being built by the French, had a different design to the Soviet reactor, much more like the French-built reactor Osiris, with features added from the reactor at Institut Laue-Langevin (ILL), Grenoble. I was shown around by the reactor manager, Dr Zaki, and allowed to look at drawings of the reactor, but not given copies. Therefore I illustrate the reactor using drawings of the Osiris facility, with the modifications I know about superimposed (Figs 2, 3). These drawings come from an article in the December 1981 *IAEA Bulletin* which described the safeguard arrangements.

The reactor is, or was, a light-water moderated swimming-pool type. This makes use of a large open pool of water about the size of a small deep swimming pool, with the reactor internals submerged therein. This would run at 70 MW under normal circumstances, but the Osirak reactor has a heavy water (D_2O) moderator attached which keeps the power to be kept down to 40 MW. (There was confusion on

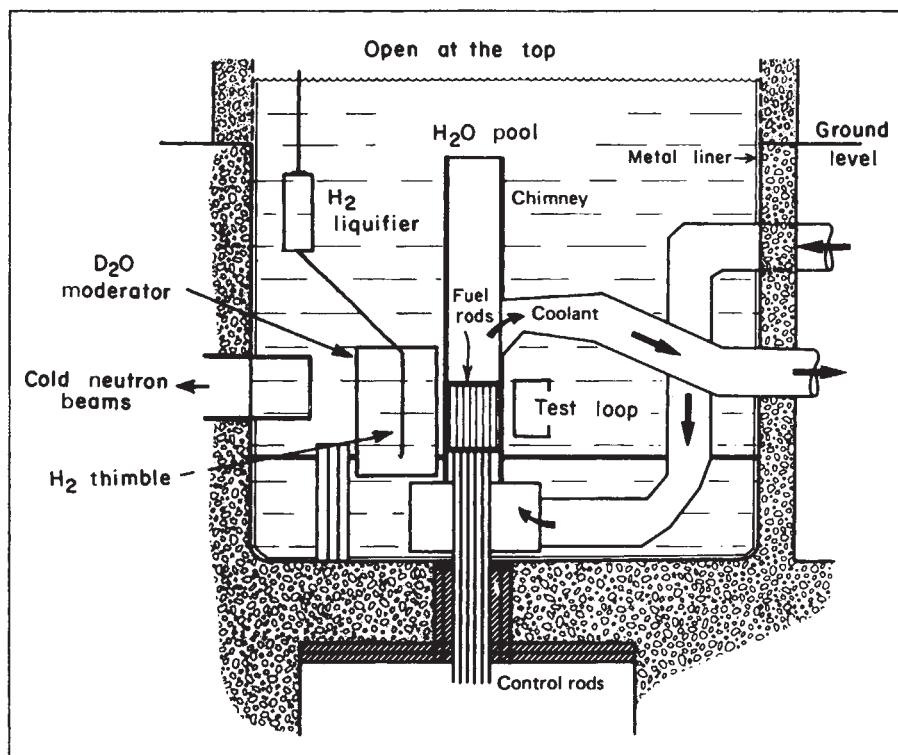


Fig. 3 Vertical section of the Osirak-type reactor.

this point in the world press in June 1981.) At 70 MW, the neutron flux of Osiris is said to be $4 \times 10^{14} \text{ n cm}^{-2} \text{ s}^{-1}$ and presumably at 40 MW is proportionately less. The fuel was to be in separate fuel pins with 95% enriched uranium. A full load of fuel pins weighs 12 kg.

In the D_2O moderator is a small liquid hydrogen thimble. This thimble slows down the neutrons by collisions with the cold hydrogen, and therefore produces long wavelength beams for neutron beam research. At ILL in Grenoble a 30 litre liquid deuterium moderator is used for the same purpose. Deuterium is preferred for large moderators, and prevents capture on hydrogen, but the collision length on deuterium is too long for small moderators.

It was unclear exactly what the Iraqi centre intended to do with these beams, but I understand that the plan was to be made by Dr Ja'afar. I mentioned that my choice would be to produce one polarized neutron beam, using a supermirror polarizer of the type designed by the Hungarian physicist, Mezei, and one beam for neutron diffraction research. The 2-axis spectrometer for neutron diffraction could be moved over from the 10 MW Soviet built reactor. At present the Iraqis are not using this spectrometer because the beam is smaller than those available in other parts of the world so that the experiments had become uncompetitive. The use of a higher flux of neutrons and the use of cool neutrons would make it equal to any in the world.

The Israeli attack

The neutron beam from the cold thimbles and from a hot source were to pass into a

rectangular neutron beam hall covered with a bridge crane. This is reminiscent of the neutron beam hall at ILL, but unlike that at ILL which is on the surface, is underground with shielding on top — about 2 feet of concrete and 4 feet of earth. In June 1981 a bomb hit the side of this hall and destroyed it, causing the crane to fall in. However, at that time no experimental equipment had been moved over from the small reactor, and a scientist who was working there was not killed.

As far as one can understand the garbled "justification" for the air raid provided by the Israeli Government, it was believed that this hall was for plutonium production. Yet the neutron flux here would be much too small for that purpose since it is some distance from the reactor; the flux of cold neutrons for experiments can be kept high by total external reflection from a neutron guide — as at ILL — but the cold neutrons are only a small fraction of the total. Typically a cold neutron beam would be 10^{10} n s^{-1} over 100 cm^2 compared with a hundred thousand times larger inside the reactor.

On the side of the reactor opposite the D_2O moderator is space for engineering tests; a pressurized water reactor (PWR) loop for test, which is almost a separate reactor on the side. This loop was also bought from France. The PWR loop was a single 15×15 fuel bundle of less than full length. The spent fuel can be taken through a dam into a channel and thence into a hot cell where it can be cut up. Several holding tanks exist for the radioactive material. Also on this side is a small 0.75 MW reactor which is essentially a mock up of the core. (This is called Tammuz I; the main reactor

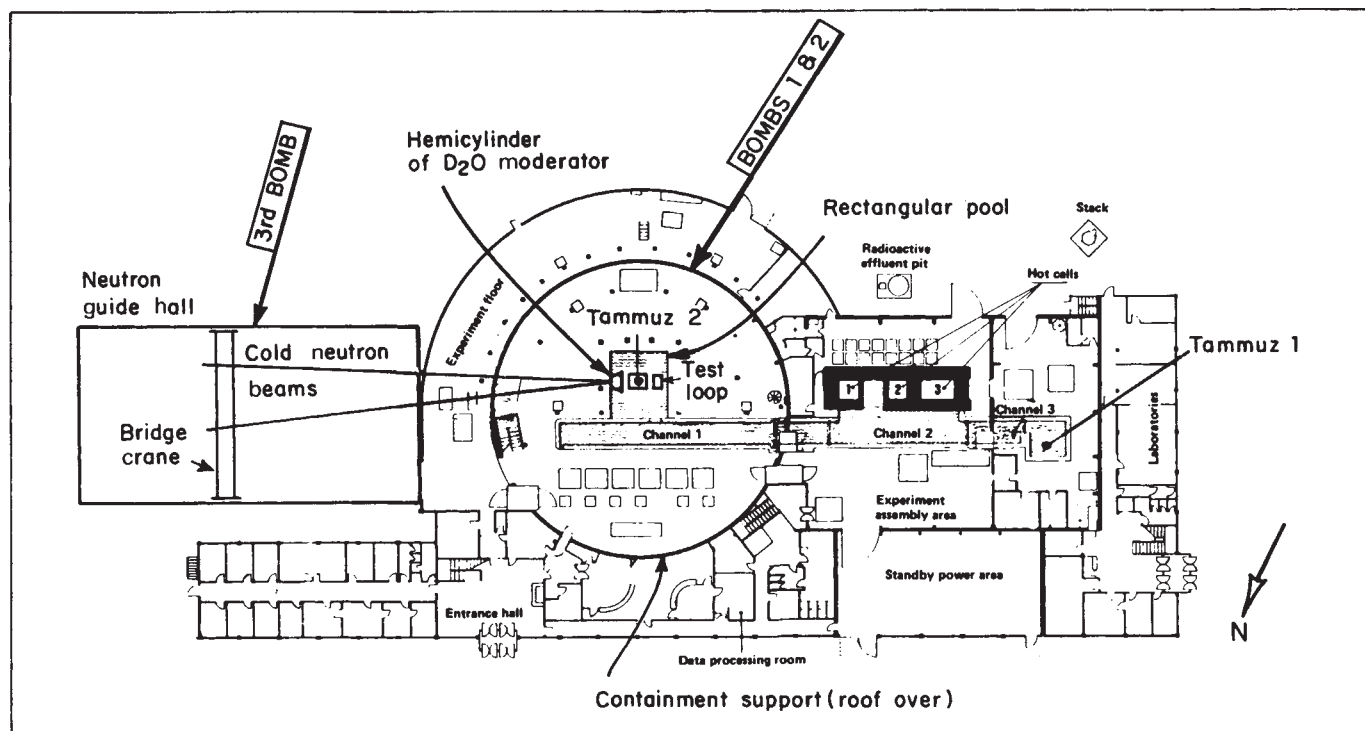


Fig. 2 The internal layout of the Osiris-Isis research complex at Saclay, France, with modifications superimposed to show the Osirak Tammuz 1 and Tammuz 2 configuration.

is called Tammuz 2.)

The containment vessel of the reactor has been destroyed. Apparently one bomb hit from the south and opened a hole; a second bomb came through the hole and exploded inside. This second explosion also destroyed the control room where one man was killed. A large part of the containment and upper floor fell into the swimming pool of the reactor, but the reactor internals were covered with water (about 30 feet), which cushioned the shock. The fuel pin supports seem undamaged. So also are the welds of the pool itself. The hydrogen liquefier (for the cold thimble) which is above the reactor level is also undamaged. Of course, for any safe restart, all welds will have to be retested but they seem intact. Apparently no water leaked out of the tank. This shows, *inter alia*, that if the reactor had been operating when the facility was bombed, no radioactivity would have been released. This should reassure those who believed that bombing a reactor automatically leads to radioactivity release.

The control rod drives are in a small cramped room underneath the reactor and are accessible in operation. They are in a region of low neutron flux during operation. They are untouched by the explosions. So also are various pumps, electrical transformers and circuits.

Connected to the reactor building on the north is an office and a laboratory building. On the south-east of the reactor is a separate large building with machine shops. On the south-west of the reactor there is a radioactive waste facility under the direction of Dr Schemmel. This facility was also bought from the French, and is for

training in encapsulation of radioactive waste in concrete and other treatment of waste.

Further away from the reactor, in a separate building to the west, is the so-called "Italian project." This is a fuel fabrication plant, directed by Dr Al-Jarah and built by an Italian firm, SNIA, as contractor. This is, or was, to make UO_2 ; to go on from there, to fabricate pellets by sintering and pressing and placing them in zircalloy cladding. Much of this is done in special, clean rooms. There exist autoclaves for cutting, and facilities for redissolving fuel pins that are badly made and starting again. This was not a large facility, only enough for a few bundles. It was planned to use a few hundred pounds of uranium for this process. There is equipment for testing the zircalloy surfaces and a Jarrell-Ash optical spectrograph.

It is claimed that the radioactive waste facility, the hot cells, and the fuel fabrication were for trainign personnel in these functions so that they could back up a nuclear electric power programme.

In 1975 Iraq embarked on an ambitious nuclear power programme. They intended to buy four nuclear plants from the West, one of which should have been operating by 1982. This programme was clearly excessive. The electricity consumption of Iraq is only a little over 4,000 MWe, and attaching a 1,000 MWe electrical generating plant to this grid seems marginal (the standard electrical engineer's rule is that no power plant should be more than 10% of the grid). Moreover, known reserves of oil in Iraq are large and the country has not been completely explored. What discouraged the Iraqis, however, was the high prices of

the bids by Western firms for constructing the power reactor. As a result no actual power plants were begun. Now, with the war with Iran in progress, Iraq cannot ship oil through the contested Shatt-al-Arab and Syria has closed its frontiers, thereby closing the oil pipeline to Tripoli and the railroad to Istanbul. Even though Saudi Arabia and Kuwait provide \$4,000 million and \$2,000 million a year respectively in subsidies (officially loans to be replaced by oil sales later), Iraq can now only start a few capital projects, and must choose them carefully. I was told by others that a \$1,000 million project for redoing the sewage system of Baghdad was suspended on 15 December 1982. The nuclear power programme therefore makes even less sense than in 1975.

International cooperation

I do not have the expertise to know whether these facilities are needed in a nuclear power programme. Nor do I know whether they are useful for plutonium reprocessing for bomb making. The cost of the research and training facility is small compared with the total cost of the nuclear power programme. It would seem useful to train local engineers and scientists who can recognize problems with fuel pins as they arise, and can fix leaks without having to resort to Western consultants. This, in any event, is the approach taken by India and being taken by Egypt.

I suggested to the Iraqis that they collaborate with scientists at CERN at Geneva, and at ILL in Grenoble.

Three years ago the physicists at Tuwaita began a small collaboration with scientists at CERN. This was started by Dr

Ja'afar, who has not now visited CERN for 3 years. In summer 1981, Dr Salman and his wife visited CERN. In late June, Dr Salman fell sick with a paralytic ailment. CERN scientists immediately suspected thallium poisoning which was the cause of death of two Iraqis in London 5 years ago, but the Geneva physicians could find no trace. Dr Salman was getting better when he developed jaundice and died. No autopsy was performed and his wife took his body back to Baghdad the next day.

Dr Ghofar, vice chairman of the Iraqi Atomic Energy Commission, told me that although no one knew the cause of Dr Salman's death, no scientist from Tuwaitha felt inclined to collaborate with CERN. This was on 28 December, 1982. In January 1983, two Iraqi scientists visited CERN again.

Inspections

The world, and particularly Israel, is interested in knowing whether the existence of these facilities in Iraq is an aid to a programme for making nuclear bombs. This has been the subject of many memoranda and reports from the IAEA, and in particular a report by the Director-General, Dr S. Eklund, to the UN Security Council on 19 June 1981, and an article by Dr Gruemm in the *IAEA Bulletin* in December 1981. There are four major items: possible diversion of uranium-235; possible production of plutonium; use of the reactor for training; and later conversion of the facilities. I will discuss these in turn; Iraq has signed the NPT, and has undertaken not to make or acquire nuclear weapons and agreed to allow its facilities to be inspected by IAEA.

The reactor vessel is open at the top as shown in Fig. 2. This makes inspection of the reactor particularly easy. First, it is possible not only to see the reactor core that is in use, but also the used reactor cores. The radioactivity of the fission products produces Cerenkov light in the surrounding water, and it is therefore easy to be sure that there are no dummies.

One fuel load contains 12 kg of uranium enriched to 95% in uranium-235. It is barely possible to remove this fuel from the reactor and separate the uranium chemically. But a "significant quantity" which as defined by IAEA is the amount for one bomb or 25 kg, is twice the amount available at one time. It is intended that fuel cores only be provided from France as needed. Since diversion is easily seen, the supply of fuel cores would be cutoff (from both Tammuz and the Soviet reactor) and a significant quantity would not be accumulated without being observed.

Every 2 weeks it was intended that an IAEA inspector should examine the reactor to ensure that the fuel was still in place, and cameras were to examine it in between. Moreover, French technicians were to remain at the reactor continuously, providing daily inspections. I proposed to the Iraqis that distinguished visitors should also be

asked to inspect the reactor.

Second, there are a number of reasons why the Tammuz 2 reactor is not very suitable for plutonium production. It is a light-water moderated reactor, and there are no spare neutrons to produce plutonium. The plutonium production rate would be about one third of the rate of destruction of uranium-235. Since the reactor runs on separated uranium-235 it would be better to take the uranium-235 itself for a bomb. But as noted above there is not enough uranium-235 in one fuel load and attempts to collect several loads would be seen by inspections and prevented by international cutoff of fuel supply.

The reactor is very different from a heavy water moderated reactor of the Chalk River NRX type, or Savannah River type, which can run on natural uranium, and has neutrons to spare to produce plutonium. The Dimona (Israeli) reactor is of this type, so Dimona is a good reactor for making bomb grade plutonium but Tammuz 2 is not.

Third, there is, of course, no doubt that training in handling radioactive materials in reactors and in industrial procedures generally is useful for anyone who wants to build and operate a chemical reprocessing plant to separate plutonium from uranium in reactor spent fuel.

Fourth, there is also no doubt that the existence of a laboratory building, with machine shops and facilities, is useful in preparing for any enterprise. But exactly how useful? Provided that international inspection continues there is no obvious danger of the Iraqis collecting a significant quantity of uranium-235 or producing plutonium in a significant quantity. However, any country is entitled to withdraw from the NPT at 3 months notice and the treaty is coming up for review in 1985. At the 1980 review in Geneva, more than seventy countries refused to discuss tightening inspection procedures because the large countries, the United States and Soviet Union in particular, daily violate the treaty by continuing the nuclear arms race, and are also not helping the nuclear power programmes of the small countries.

Potential for bombs

In order to estimate the value of these facilities, I have tried to imagine myself in charge of making nuclear bombs in Iraq. I would try to make best use of the facilities available to me. If Iraq had not withdrawn from NPT, I would not know how to use the Tuwaitha facilities without an overwhelming probability of being found out. So, I would build a separate secret facility in another place. Since the very existence of this facility would be a violation of the IAEA regulations under NPT, it would have to be very secret indeed.

If Iraq abrogated the NPT treaty, or if the treaty were abandoned by the world because of lack of confidence in the United States and the Soviet Union, I would have more free rein. Then I would convert the

swimming pool reactor which now operates with ordinary water into a heavy water reactor; I might buy the heavy water in advance, though I would have to declare it to IAEA, so the world would be alerted to my intentions. Or I might make heavy water in a specially designed plant. I would arrange that I had access to a supply of natural uranium (not restricted though reported to IAEA) and I could use experience gained at Tuwaitha to make fuel rods. These fuel rods would have to be changed once a week to remove the plutonium-239 before the concentration of plutonium-240 built up. Then the hot cells would have to be modified for a high throughput, or operated without regard to the radiation dose to personnel.

These could all be done, but the changes to the existing arrangement would be considerable, and it is arguable that it would be simpler to start from scratch. I note that Pakistan has almost certainly been engaged in making a bomb since 1974. At that time India exploded a bomb and President Bhutto telephoned Dr Munir Ahmed Khan, then in Vienna, to ask him to return to Pakistan as head of the Atomic Energy Commission. Pakistan has *not* signed NPT.

Peaceful intent

I therefore estimate that the existence of the Iraqi nuclear research centre would give them at most 1 year's start in a 10-year programme to make bombs. This year's start would be compensated by the fact that the world would be warned by the obvious change in the programme and could hinder it if they chose.

One hundred and seventeen nations, including the United States, have signed the NPT and by doing so agree that the present procedures to prevent proliferation of nuclear weapons are reasonable. However, the world has not decided how to behave towards those countries who, by not signing, have expressed their disagreement, especially that country which expresses its disagreement violently. The matter is urgent, because the 1985 NPT review is almost upon us.

The vice chairman of the Iraqi Atomic Energy Commission, Dr Ghofar, and two commissioners, Dr Kittel (a physicist) and Dr Al-Mallah (a PhD in nuclear engineering, University of Wisconsin), welcomed me to Iraq and to the centre and asked me to tell the world what they are doing. They have not yet decided whether to rebuild the reactor. This may be because they cannot get agreement with the French. Sa'adallah Al Fathi, of the Iraq Petroleum Refineries Administration, knows nothing about the project, but is insistent that national pride demands that the reactor be rebuilt. If they decide to do so, I urge the Iraqis to set up an international advisory committee, like advisory committees in the West, made up of people of many nations. This would help the Iraqis in their research and help satisfy the world of their peaceful intent. □

Uncertainties about uncertainty principle

Is the notion that successive measurements on an isolated quantum system will yield the same result an axiom of the quantum theory or a consequence of it? This old argument has now resurfaced.

THE paradoxical nature of the quantum theory has since the mid-1920s been neatly encapsulated in Heisenberg's uncertainty principle. For why should it be that, in the real world, it is impossible to make simultaneous measurements of, say, the linear momentum and the position of a particle? Or of all three components of its angular momentum? The simple and familiar answer to all such questions is flatly to assert that this is what the quantum theory has shown the real world to be like, but this, of course, is unconvincing. The uncertainty principle is paradoxical because it conflicts with expectations derived from experience with macroscopic systems with the result that discussion of the uncertainty principle has always been embedded in a rich matrix of explanation, or at least of *post hoc* justification. And the process continues.

De Broglie's argument is the oldest and that most often found in elementary textbooks: once it is accepted on the basis of, say, electron diffraction phenomena that particles can also be represented as trains of waves, it is natural to expect a trade-off between the localization of a particle represented by a wave-packet and the spread of wave-number (representing momentum) of which it is constituted. In the 1920s, however, Dr Broglie's argument was too slight to banish the sense of paradox, while the need for understanding these problems was urgent. This was the task undertaken by Niels Bohr and the Copenhagen school, with its doctrine of complementarity, the definition of what is meant by a quantum state and the whole vocabulary in which quantum theory is even now discussed.

Inevitably, the Copenhagen school has become the focus of the discontent of those who may be called the dissidents. Out and out dissenters, those like the later Einstein who held that the uncertainties of the quantum theory will at some stage melt away regard the whole Copenhagen episode as a gigantic aberration, as when there were popes based at Avignon.

M. Cini, of the University of Rome (*Il Nuovo Cimento* 73, 27; 1983) has a more subtle but more interesting complaint against the Copenhagen legacy. What, he asks, is the logical status of the belief that if you make a measurement of some physical property of a quantum system, supposed to be at the outset in some uncertain stage, and discover some specific value of the physical variable concerned, then (other things being equal) the system will be found

to persist in a condition in which the physical property remains unchanged? Cini's question goes to the root of the Copenhagen argument. His answer is comforting — complementarity survives — but some puzzling features of the argument about the effects of measurements on quantum systems are neatly clarified.

Here is an example, one of Bohr's *Gedankenexperimenten*. Take a screen in which two parallel slits A and B have been cut and conceal a source of electrons behind it, symmetrically in relation to the slits. If the source emits photons, to which the argument equally applies, the result is Young's apparatus for the demonstration of optical interference. In other words, detectors on the side of screen opposite from the source can be used to show that there are some points at which the chance of the arrival of an electron is literally zero and in a statistical sense, the standard interference pattern is recovered by the detector even when particles (or photons) are emitted singly, one by one. So here is another paradox: since the interference pattern is destroyed if one slit or the other is covered up, there is a sense in which each particle reaching the detector must be held to have passed through *both*.

How can this be accomplished? Formally the procedure is straightforward. One definable state of the system is that in which the particle travels through slit A, another is that in which it travels through slit B and, if each of these is represented by some symbol (ψ_A and ψ_B after Schrödinger or $|A\rangle$ and $|B\rangle$ after Dirac), then the state in which the particle travels through both slits is that represented by the sum of the two symbols multiplied by some number (generally complex). The outcome is that it is possible to record the arrival of single particles in the plane of the detector, and indeed by repeated measurement to reconstruct an interference pattern from which the momentum of the particles can be derived, but only at the price of being entirely uncertain about the routes, slits A or B, by which the particles have travelled. But why not cheat by equipping slit A, say, with another detector that will tell whenever a particle passes through? Because that measurement will disturb the state of each particle, so that each will finish up in a state represented by some other linear combination of the separate slit-state symbols than the simple sum, whence the notion that measurements affect the states of quantum systems as if they

were operators operating algebraically on the individual states.

Even in retrospect, it is quite remarkable that in the 1920s the entire algebra of the quantum theory was reconstructed from such simple considerations. But Cini's complaint centres on an assumption made then and afterwards — that the effect of trying to cheat as described above is to convert the composite state of a particle into one or other of the elementary states of which it is composed. Thereafter, the argument goes, further measurements designed to tell which slit has been traversed will yield the same result.

Cini's argument is that this assumption, while in practice valid, is logically unnecessary. Rather, he says, the almost but not quite exact projection of the composite state vector onto just one of the components of which it is composed is a consequence of the way that measurements are made. For this purpose, he has devised a *Gedanken*-detector for particles, a model of a proportional counter made of idealized ionizable atoms which are mathematically simple enough for the detector and the particle to be treated as a whole. The objective is to calculate the time evolution of the combined system. Predictably, when the number of atoms is large (or the counter macroscopic), there can be only small departures from the rule that performing a measurement on a quantum system forces it into a state corresponding to the measured value.

So what? Bohr in the 1920s was fully aware of the need that an effective measuring device should be a macroscopic system, while much of what Cini has to say is for practical purposes contained in the formulation of quantized statistical mechanics by people like Leon Rosenfeld in the 1930s. Cini, however, has two axes to grind. He wants the projection postulate eliminated from the quantum theory because it is logically unnecessary, and he wants to deny descriptions of the quantum theory that take forms such as "measurement disturbs the system measured". Not a bit of it, he says; the system and the instrument are part of a system that may be self-contained, and which is evolving under the influence of their mutual interaction. A simple conclusion? Not all of Cini's referees have shared this view, with the result that the editor of *Il Nuovo Cimento* has given him the luxury of a "note added in proof" that runs to more than 2,000 words to defend himself against his silent critics. [1]

Cell division

Coordinating histone transcription and DNA replication

from Paul Nurse

ONE of the more intriguing puzzles posed by the cell division cycle is how the various periodic processes required in the cycle are temporally coordinated. A good example is the coordination of histone supply with DNA replication, since in most eukaryotic cells histone protein synthesis appears to be confined to S phase¹. This is true of the budding yeast *Saccharomyces cerevisiae*², and a series of recent papers from Hereford and her collaborators have provided considerable insight into how DNA replication and histone synthesis are co-ordinated in this organism.

Control at the level of transcription of the histone genes appears of major importance in maintaining coordination. This was established by using cultures synchronized with the yeast pheromone α -factor, which blocks cells in G₁, and cells fractionated by elutriation centrifugation according to size, and thus position in the cell cycle³. Monitoring the histone H2A and H2B transcripts by northern blot hybridization and the H3 and H4 transcripts by *in vitro* translation showed that all histone messages reached a peak level during S phase. That the periodic accumulation of histone messages is the result of increased histone gene transcription was demonstrated by pulse-labelling α -factor-synchronized cells and hybridizing the labelled RNA to H2B DNA immobilized on a filter⁴. The rate of H2B transcription peaks at the G₁-S phase boundary and can account for the peak in the level of H2B transcripts seen a little later in mid-S phase.

A second level of regulation operates post-transcriptionally. When DNA replication is blocked using a temperature-sensitive mutation in *cdc8*, a gene function required for DNA synthesis, then the turnover of H2A and H2B transcripts rapidly increases³. This produces a rapid fall in the levels of histones, as has been shown when DNA replication is blocked using hydroxyurea². The results suggest that there are two major controls which coordinate histone supply with DNA replication. First, there is an activation of histone gene transcription at the G₁-S phase boundary, and second, there is a stabilization of histone messages by the process of DNA replication itself. The two controls would result in a sharp increase in the amount of message at the beginning of S phase, followed by a rapid drop at the end of S phase when DNA replication ceases and turnover of histone messages consequently increases.

Paul Nurse is in the School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG.

Two elegant papers from Hereford's laboratory have given more information about the mechanism of activation of histone gene transcription. In one paper, the point in the cell cycle at which transcription is activated has been defined precisely using a *cdc7* temperature-sensitive mutant which blocks cells in late G₁ (ref. 4). Cells synchronized with α -factor and then arrested at the *cdc7* block point show an increase in the rate of H2B transcription even though DNA replication is not initiated. It is thus traverse through a late stage of G₁ which activates histone transcription, rather than the onset of S phase. Furthermore, in the cells arrested at the *cdc7* block point the rate of H2B transcription remains at a high level, suggesting that traverse of a later stage in the cell cycle is required to turn transcription off. This stage must be before the mid-S phase block point imposed by a *cdc8* temperature-sensitive mutant since in these arrested cells the rate of H2B transcription shows the normal pattern of a peak followed by a fall to a low level. This behaviour would be expected if histone transcription ends with the first third of S phase.

In the second paper, a DNA sequence next to the H2A and H2B genes is shown to be necessary for periodic transcription during the cell cycle⁵. The H2A and H2B genes are adjacent, but separated by a spacer region of about 800 nucleotides in which transcription of both genes is initiated. First, the H2A and *lacZ* genes were fused to construct a gene coding for a hybrid protein made up of the first part of H2A histone and β -galactosidase, and under H2A transcriptional control. A plasmid containing the H2A-*lacZ* fusion gene along with the 800 nucleotide spacer region and part of the H2B gene was then integrated into the chromosome at the site of the *leu2* gene. Cells synchronized with α -factor and containing the integrated plasmid synthesized β -galactosidase at a low level but synthesis did not change periodically during the cell cycle. When the experiment was repeated with a plasmid containing an extra 2.3-kilobase fragment encompassing the rest of the H2B gene and some sequences flanking its 3' end β -galactosidase was synthesized at a higher level and periodic variations were seen with peak activity at the same time that H2B transcripts peak in amount. Clearly a DNA sequence located at the 3' end of the H2B gene is required for regulation of H2A gene transcription in the cell cycle.

The same sequences also contain an *ars* (autonomous replicating sequence) activity, the presence of which enables the

plasmid to transform yeast cells at high frequency. Deletion mapping has shown that *ars* activity and the effects on β -galactosidase synthesis must be within 50–100 nucleotides of one another. As *ars*-containing plasmids can replicate in the yeast cell the *ars* sequences would seem to contain a chromosomal origin of replication⁶; if so, then the sequence required for periodic histone transcription might also be a replication origin. Osley and Hereford suggest that the changes in chromatin structure that are required to initiate DNA replication at the beginning of S phase also activate histone transcription, and that activation of the replication origin once every cycle could be responsible for the periodic synthesis of histone mRNA.

Although the identification of *ars* sequences with replication origins remains uncertain, this does not affect the main conclusion that a particular sequence is required for periodic histone transcription, and that the sequence can respond to the changes in chromatin structure that take place when yeast cells traverse the cell cycle. Folded chromosomes (similar to nuclear matrix preparations only isolated in low salt) sediment differently in cells blocked at different stages of G₁, and at stages from G₁ through S phase to G₂ (ref. 7), and there are also alterations in the association of plasmid with folded chromosomes which occur early during the cell cycle⁸. Some general change obviously must occur in chromatin state in late G₁ — perhaps a gradual decondensation or an altered binding to nuclear matrix — and could influence the histone-associated *ars* so that it activates histone gene transcription.

Such a control mechanism may be used quite generally to regulate expression of genes whose products are required in late G₁ or S phase; indeed, there is some preliminary evidence indicating that the transcription of the HO gene involved in mating-type switching may be regulated in a way similar to the histone genes (K. Nasmyth *Nature*, in the press). It is also possible that the same control mechanism is used in other eukaryotic cells since in most cell types histone gene transcription usually only occurs during S phase, and there are considerable changes in chromatin structure as cells traverse G₁ and initiate DNA replication¹. Monitoring the behaviour of the *S. cerevisiae* histone gene fusions in other eukaryotic cells will make it possible to test this possibility. □

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Prehistory

Thoughts from artefacts:
towards a cognitive archaeology

from Alun M. Anderson

IN a recent publication*, giving the text of his inaugural lecture as Disney Professor of Archaeology at Cambridge University, Colin Renfrew argues for the opening up of a new field of 'cognitive archaeology' — an archaeology which would draw on such diverse subjects as ethology and artificial intelligence as well as those more traditionally associated with archaeology. The central point of his argument is that the archaeological record contains information not only about human technology, economy and society, but also about human reasoning, the concepts and ways of thought shared by the citizens of a particular culture.

What particularly emboldened Renfrew to suggest that a cognitive archaeology might be possible is the recent, and successful, emergence of social archaeology. It was only some twenty-five years ago that it was argued that a social archaeology would have 'no sound intellectual basis at all', since 'historical events and essential social divisions of prehistoric peoples don't find an adequate expression in material remains, it cannot be right to try to arrive at a knowledge of them by archaeological interpretation'. As it turned out, social archaeologists did find ways of revealing something of the social structure of societies from archaeological remains.

A good example, described by Renfrew, comes from the study of geographical patterns of early settlements. Using as data the sizes and locations of settlements revealed by archaeological survey, it is possible to recognize hierarchical patterns that indicate the political geography of the area at the time in question. The validity of the technique can easily be seen by applying it to the present pattern of towns and cities — a political map of modern Europe emerges. The recognition of hierarchy at this level immediately suggests a corresponding social hierarchy; of 'central persons to accompany the central places'.

A second example comes from the study of cemeteries where it is possible to examine the goods accompanying each individual burial. Obviously, the distribution of goods according to sex, age and so on can lead to a good idea of the social structure of the community from which they came.

In some senses social archaeology is on the road towards cognitive archaeology, for it is reasonable to infer that the social divisions of a society reflect, or are reflected in, corresponding concepts about that society. Obviously though, attempts

to understand the modes of thought and beliefs of a vanished society are going to present special problems of their own, which direct comparison with anthropological studies of living groups do not in themselves solve. Renfrew is optimistic that sets of analytical procedures are possible which are not simply based on an 'inspired exercise of the creative imagination' like so many commentaries on ancient art. Certainly, going further back in time, much has already been accomplished in recording the emergence of characteristics that are believed unique in man. These include the use of tools, and perhaps language, with *Homo habilis* at around 2 Myr BP; the use of pigments (perhaps implying body decoration) with *Homo erectus* at 1.5 Myr BP; and, with *Homo sapiens*, the use of fire, development of specialized 'tool kits', apparently deliberate disposal of the dead, cave painting, the making of repeated marks in a manner indicative of counting; and then, later still, the emergence of agriculture.

A problem remains, however. To what extent does the emergence of a particular behaviour — even an apparently intelligent one — tell us about the cognitive processes that produce it? We only have to go back to the turn of the century to see the 'agriculture' practiced by some species of ants, and the navigation and homing abilities of insects and birds, used as evidence of 'intelligence' in animals. What the then new science of ethology did was to show that apparently sophisticated behaviours can be based on very simple models of the world. Indeed, there are many archaeologists who would favour the view that farming arose through a trial-and-error process which tells us nothing of man's cognitive abilities.

An important distinction can, however, be drawn, for the archaeological record contains not just information about 'doing things' but also of 'thinking about doing things'. A clear example is seen in the carefully made stone cubes found in the Mohenjo-daro site of the Indus Valley civilization. The cubes have weights that would be produced by multiplying a constant unit of weight (actually 0.836 g) by such integers as 1, 4, 8 up to 64, then 320 and 1,600. This is purely archaeological evidence, but it allows us to assert, not just that the society had a concept of weight, units and numeration, but surely that the society had a concept of 'weighing'; that is, of equivalence of weight among objects made of different materials, and hence notions of 'value' and of rates of exchange between commodities. An important difference emerges here in the representation (or 'mapping') of weight and the 'map-

ping' seen in the animal world, as exemplified in the dance language of the bee. It is only in the former case that a mapping device — the system of weights — has been constructed.

Clear archaeological evidence for deliberate action carried out to a preconceived design is also revealed in the planning of towns; the streets of Mohenjo-daro, for example, are laid out on firm rectilinear principles. Caution will be needed in interpreting evidence of planning, for many simple rules of organic growth can result in symmetrical designs, and apparent order in town plans can emerge without plan at all; but these do not seem insurmountable obstacles to research.

Renfrew appears at his most cautious when he discusses attempts to infer religious practice from archaeological finds — an area where the non-archaeologist might feel that the archaeologist is most free to speculate on the thought processes and beliefs of ancient cultures. Perhaps it is abuse of that freedom that makes Renfrew argue for efforts to find a much more rigorous methodology for the study of religion from material remains of religious practices. Indeed, even in the case of cave art, we have no clear and unequivocal method of demonstrating that a given form is a depiction; there are numerous natural forms that mimic the creations of man.

What Renfrew is really after is a coherent body of theory which will allow inferences about the cognitive processes of past societies to be made without, as he says, 'dizzy leaps'. But how are cognitive processes to be categorized and described? Ethology and artificial intelligence, on which Renfrew hopes to draw, are full of cautionary tales. Numerous attempts have been made to categorize 'cognitive' or learning 'abilities' among diverse animal groups and to set up phylogenetic levels of 'intelligence'. All have failed to overcome the problem that each species learns in its own way, a way that is adapted to the particular environment in which it lives, so that no general categories of learning ability emerge. Again, studies in artificial intelligence have shown us the difficulty of classifying tasks without knowing exactly how they are carried out; it is often the apparently simple skills that require the most complex computations.

Renfrew is aware of these problems but remains optimistic that 'thoughts' do find effective expression in the archaeological record and ways can be found of studying them. In the long term he may well prove right, but, at least for the time being, cognitive archaeology may have to be content, in Renfrew's words, to 'tread an uneasy path between the pretentiously jargon laden and the blindingly obvious'. □

Alun M. Anderson is the editor of the News and Views section of Nature.

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Geophysics

Tracing water flow in the ocean

from Wolfgang Roether

CHLOROFLUOROMETHANES (CFMs), the gases used in refrigerators and aerosol cans, have recently acquired a bad reputation for the effect they may have on the ozone layer of the upper atmosphere. Harmful or not, their use does provide a scientific bonus; they make it possible to trace quantitatively the exchange of water between ocean surface and ocean interior, as documented in a recent article in the *Journal of Geophysical Research* by R.H. Gammon and colleagues¹.

The work is important because the exchange of water influences climate — indirectly, by governing the rate at which the carbon dioxide from the burning of fossil fuels vanishes into the ocean, and directly, by determining the oceanic storage of heat over a time scale of some years.

Tracer substances like the CFMs have the unique advantage that their dispersion within the ocean will be directly analogous to that of CO₂ and heat. The measurements made by Gammon *et al.* in the north-east Pacific show, for example, near-constant values in a surface water layer 100 m or so deep, and beneath it an exponential decrease with depth to vanishing concentrations below about 600 m. This finding gives immediate proof that little fossil-fuel CO₂ could have penetrated beyond 600 m depth in that part of the ocean, over the few decades that both CFMs and fossil-fuel CO₂ have been present in the environment.

CFM is not the only man-made substance to be inadvertently released into the environment and used as a tracer of the movement and mixing of water in the ocean. The distribution of tritium, a by-product of the atmospheric nuclear weapon testing of the 1950s and 1960s, has been measured worldwide^{2,3}, and results similar to those given in Gammon *et al.*'s article were reported a decade ago⁴.

Oceanic CFM measurements are, however, rather more special. The main reason for this is that the detection of tritium, at oceanic concentrations of 10⁻¹⁹ (mol tritium per kg of water) or less, though possible by its radioactive decay, is difficult and can be performed by only a few land-based specialized laboratories. Oceanic CFM concentrations, at or below 10⁻¹², on the other hand, can be measured with a gas chromatograph thanks to the invention of the electron capture detector by the UK scientist J.E. Lovelock⁵. This has made possible easy and low-cost oceanic CFM measurement. All that remains is its perfection — with contamination from CFM use as the remaining problem.

Analysis on board ship, as Gammon *et al.* rightly point out, allows measurement immediately after bringing up water samples from depth and can thus guide further sampling effort during an oceanographic cruise.

Oceanic tracers have helped build-up better numerical ocean models. The oceanic distribution of the tracer represents the ensemble of water flow trajectories from the ocean surface where the tracer material entered from the atmosphere, integrated in time to the respective point in the ocean where the tracer is sampled several years later. The tritium and CFM distributions are both transient in nature, depending on

the history of their release into the environment, and are therefore very different from that of salinity, for example. From the available tritium and CFM observations, it is apparent that there are significant differences between their distributions, and both exhibit a remarkably clear structure. In future modelling efforts it may be possible to use both CFMs and tritium, and perhaps further tracers⁶, to produce a combination which gives particularly powerful observational constraints on ocean movement and mixing at upper and intermediate depths. □

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6. A major effort to collect such tracer data is now underway, for example, in the US Program Transient Tracers in the Ocean.

Earth science

Kimberlites revisited

from Chris Hawkesworth and Martin Menzies

THE volcanic rocks known as kimberlites, famous for the diamonds found in them, have long been important in the study of the upper crust. They have their origin some 200 km deep down in the mantle¹ and, fortunately for the Earth scientist, as they ascend from these great depths, they bring to the surface a sample of the rocks of the uppermost mantle and the continental crust.

So far, detailed geochemical analysis has been of these inclusions, or xenoliths, rather than of the kimberlite itself, for the kimberlite is very susceptible to near-surface alteration. Now this has all changed as underground samples of fresh kimberlite have become available; new work reported by M.T. McCulloch *et al.*² in this issue of *Nature* (p.400) and by C.B. Smith (*Nature*, in the press) shows that at least some forms of kimberlite provide potent evidence for the existence of an 'old' trace-element-enriched upper mantle.

Two important conclusions have emerged: first, there are kimberlites and kimberlites. Most of those analysed previously were non-micaceous, or 'basaltic' kimberlites which generally have low ⁸⁷Sr/⁸⁶Sr and high ¹⁴³Nd/¹⁴⁴Nd ratios^{4,5}, similar to those of many ocean island basalts. Micaceous kimberlites and lamproites, by contrast, have much higher ⁸⁷Sr/⁸⁶Sr and lower ¹⁴³Nd/¹⁴⁴Nd ratios^{2,3}, indicating they either were derived from, or at least contain a contribution from, a source region which was both 'old' and had relatively high Rb/Sr and low Sm/Nd ratios. Yet both groups are clearly derived from the upper mantle, as is shown by the presence of diamonds and mantle xenoliths; and their Nd contents, for exam-

ple, are too high for their ¹⁴³Nd/¹⁴⁴Nd ratios to have been affected significantly by interaction with the continental crust. Thus the second conclusion: low ¹⁴³Nd/¹⁴⁴Nd and high ⁸⁷Sr/⁸⁶Sr ratios, which are too often regarded as reliable fingerprints of the continental crust, do exist in some portions of the upper mantle. How then can these results be reconciled with models of the sub-continental mantle developed from studies on basalts and mantle xenoliths?

Early suggestions that the sub-continental mantle might preserve fragments of comparatively 'pristine' or 'primitive' mantle material⁶ were always likely to be greeted sceptically by the non-isotope fraternity. Particular geochemical compositions will presumably not survive for long in a convecting upper mantle, and while they may persist in the continental lithosphere, that is unlikely to be older than the age of the overlying crust. Thus continental magmatic processes probably sample both the lithosphere and the underlying convecting mantle. The former consists of old 'fossilized' mantle which, as illustrated by recent work on both mantle xenoliths^{7,8} and Karoo volcanics⁹ of southern Africa, may preserve a complex record of mantle events. In that area some relics of Archaean mantle have been recognized, but much of the lithosphere stabilized ~ 1.4–1.0 Gyr ago, at or soon after the locally widespread orogeny of the Namaqua–Natal Belt, and this was subsequently sampled by Karoo magmatism at ~ 190 Myr and remobilized by K-richite metasomatism in the

Chris Hawkesworth and Martin Menzies are in the Department of Earth Sciences, The Open University, Milton Keynes MK7 6AA.

Wolfgang Roether is in the Institut für Umweltphysik, University of Heidelberg, 69 Heidelberg, FRG.

Cretaceous⁹. Such a complex history results in a range of present-day ¹⁴³Nd/¹⁴⁴Nd and ⁸⁷Sr/⁸⁶Sr ratios generally lower and higher respectively than those of the bulk Earth, and thus contrasts with material derived from the underlying convecting mantle which is similar in composition to that sampled in many oceanic areas.

Within this simplified framework the non-micaceous kimberlites are thought to be derived from beneath the lithosphere, and they are therefore more likely to contain inclusions of high-temperature deformed peridotites and megacrysts which probably come from the base of the lithosphere¹⁰. The micaceous kimberlites and lamproites, however, contain a significant contribution from older high-Rb/Sr low-Sm/Nd mantle presumed to be within the lithosphere — and that in turn poses several intriguing questions. Do, for example, micaceous kimberlites contain fewer high-temperature xenoliths, which might be further evidence that they originated from shallower levels, or do their mineralogy and isotope ratios simply reflect scavenging of lithospheric material? What is the significance of the Proterozoic model Nd ages reported on Karoo lavas and mantle xenoliths from southern Africa⁹ and on lamproites and kimberlites from western Australia²? Were they derived from enriched upper mantle which comprised one province before the split up of the Gondwanaland, or, as McCulloch *et al.* imply, is the mantle beneath western Australia of Archaean age?

Finally, these results rekindle speculation over the role of the subcontinental mantle in models for the evolution of the crust-mantle system. McCulloch *et al.*² and McKenzie and O'Nions¹¹ suggest that recycling of old trace-element-enriched mantle from within the continental lithosphere can explain the isotope characteristics of ocean island basalts. There are problems, however, if such processes are also to account for the trace element variations in both continental and oceanic basalts. Many of the continental basalts which appear to have been derived from within the lithosphere have different trace element characteristics to those erupted in oceanic islands, and even within the oceans there is no simple relationship between isotope and trace element ratios. □

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Deep-Sea Drilling Project Leg 89

The Mesozoic superocean

from the Leg 89 staff

DRILLING from the *Glomar Challenger* in the western Pacific (Leg 89 of the Deep-Sea Drilling Project, International Phase of Ocean Drilling, October–November 1982) was planned to extend our knowledge of the geological history of what was the Earth's 'superocean' in the Mesozoic era. Most of the early record has been lost during later Mesozoic and Cenozoic time as the oceanic crustal rocks and their sedimentary cover descended into the circum-Pacific trenches. The oldest remaining part (about 160 Myr) forms the floor of the Mariana Basin. On the assumption that the basin afforded the best opportunity to sample Lower Cretaceous and Jurassic rocks, Site 585, at a point about 1,300 km east of Guam, was chosen from the results of a special geophysical survey. In two holes a sub-bottom depth of 893 m was reached. The drilling results include new information on the extent and nature of Cretaceous volcanism and the occurrence of oxygen-deficient water in the Pacific Basin.

Volcanism during the middle of the Cretaceous Period about 115–72 Myr was much more voluminous and widespread and perhaps of a fundamentally different character from Cenozoic Pacific Plate volcanism; as is indicated by the enormous amount of volcanic rock emplaced at ridge-crest spreading centres and as flows, sill and volcanic edifices (seamounts and islands). Many of these Cretaceous eruptions have patterns of distribution and ages that do not fit a simple 'hot-spot' theory — that volcanic islands (and even volcanoes on land) are erupted in linear chains as the plate drifts over anomalously hot magma-generating spots in the Earth's mantle.

The bottom one-third of the section is a series of sandstone, siltstone and breccia made almost entirely of fragments of basaltic glass and rock that formed as hot lavas erupted into the cold sea, quenching and shattering the glass. The slopes of the numerous seamounts near the basin were the sources of the hyaloclastic sediment transported by turbidity currents and debris flows onto the basin floor. Fossils deposited in the volcanogenic sediments show them to be of Aptian and Albian age (about 115–100 Myr). By late Aptian time many of the volcanoes had reached sea level and were shedding shallow-water fossils and ooids into the basin, whose floor must have been at abyssal depths given the presence of autochthonous benthic foraminifers in the sediment.

Glomar Challenger also returned to the Nauru Basin where drilling in 1978 disclosed a major section of volcanic flows of Aptian–Albian age (an earlier report of Barremian Stage fossils has been re-evaluated) and sills probably of Campa-

nian age (about 75 Myr). Re-entry into the same hole (462A) on Leg 89 encountered further basalt sheet flows and their intercalated, sparsely fossiliferous sediment layers. Reworked early Cretaceous fossils and the pattern of Jurassic magnetic anomalies indicate older sediment and Jurassic crustal rocks probably lie below the present hole depth of 1,209 m.

Deep-sea drilling in the western Pacific has added to our knowledge of mid-plate (that is, not at a spreading centre) mid-Cretaceous volcanism, not only of seamounts but also of flows, sills and volcanogenic sediments that fill basins between the groups of volcanoes. Geophysical surveys of the Mariana and Nauru Basins show that the seismic reflectors that correlate with the layered volcanogenic rocks encountered in the boreholes extend throughout the basins. A reverberant layer seen on seismic reflection profiles throughout most of the Central Pacific may show the further extent of the layered igneous rocks buried by younger sediment.

The Leg 89 scientists conclude that the layered volcanogenic rock in the deep basins is as voluminous as the coeval seamounts around then, and that neither the layered nor the edifice-building volcanism can be explained by current simple hot-spot models. Two peak episodes of volcanism are apparent. One was Aptian to Albian (115–100 Myr) and a lesser one occurred during the magnetically normal part of the Campanian (about 78–72 Myr).

The recovery of two thin bands of black radiolarian-rich sediment shows that at about 91 Myr the Mariana Basin was the site of deposition of organic carbon-rich sediments resulting from a marked oxygen deficiency in the water column. The organic carbon constitutes more than 5 per cent of one of the shales and 1.5 per cent of the other, compared with values of well less than 0.1 per cent for most pelagic sediment. The organic carbon displays kerogen with hydrogen indices of 954 and 807, and oxygen indices of 33 and 57, indicative of algae-generated organic matter which now lies on the initial part of the type 1 kerogen evolution path. The oxygen deficiency in the mid-Cretaceous Pacific Basin water-mass is a manifestation of the Cenomanian–Turonian 'oceanic anoxic event', during which carbonaceous sediments were deposited in many marine basins on a global scale. □

The Leg 89 staff included R. Moberly (Hawaii Institute of Geophysics); S.O. Schlanger (Northwestern University); M. Baltuck (Scripps Institution of Oceanography); J.A. Bergen (Florida State University); W. Dean (US Geological Survey, Denver); P.A. Floyd (University of Keele, UK); N. Fujii (Kobe University, Japan); J.A. Haggerty (University of Tulsa); J.G. Ogg (University of Wyoming); I. Premoli-Silva (Istituto di Paleontologia, Milan); A. Schaaf (Institut de Géologie, Strasbourg); R.G. Schaefer (Institut für Erdöl und Organische Geochemie, Jülich, FRG); W.V. Sliter (US Geological Survey, Reston); J.M. Whitman (Scripps Institution of Oceanography).

Astrophysics

Primordial helium abundance and big-bang cosmology

from Joseph Silk

THE primordial abundance of helium is one of the three great predictions of the big-bang theory. The theory holds that helium was synthesized during the first minutes of the expansion, and predicts its mass fraction to be about 25 per cent of the ordinary matter in the Universe. Of course, stars are also gigantic fusion reactors that convert hydrogen into helium, so it is crucial to disentangle the stellar contribution to the observed helium abundance from its primordial cosmic value. A workshop devoted to primordial helium was recently convened* by the European Southern Observatory at Garching and provided a unique opportunity to assess the current status of theoretical predictions and observations.

Over the past four years, cosmologists have honed predictions of the helium mass fraction (Y) to three significant figures. A higher-density universe synthesizes helium more efficiently, increasing Y slightly. G. Steigman (Bartol Foundation, University of Delaware) reported on the role of different types of neutrinos during nucleosynthesis. Physicists have discovered three neutrino species: the electron, the muon and τ neutrinos; and in principle, further species await discovery. Each additional species contributes to the energy density of the Universe, and thereby slightly speeds up the expansion during the high-temperature regime when nucleosynthesis occurs. As helium synthesis competes primarily with the decay of free neutrons — a neutron possessing a half life of about 10.5 min — the speed-up means that neutron capture by protons occurs more efficiently and produces slightly more helium.

Stars also produce and eject helium into the interstellar medium. Allowance must be made for this enrichment, and the primordial value of the helium abundance is denoted by Y_p . With three neutrino species, the predicted Y_p is 0.25 for a universe that is at one-tenth of the critical density for closure. In a closed universe, Y_p increases to 0.26. Only if the baryonic density is very low indeed, corresponding to a density of 1 per cent of the closure value, is Y_p reduced to 0.22. The presence of further, as yet undiscovered, neutrino species has the effect of increasing Y_p . Determination of Y_p therefore has potential implications of immense import for cosmology and particle physics.

Observers had a field day at Garching. Total failure to agree on a unique value for

Y_p left them concluding that any value between 0.22 and 0.26 was consistent with their data. The problems began surprisingly close to home, with the Sun. D. Gough (University of Cambridge) reviewed the remarkable results of solar seismology. Observations of solar oscillations probe the structure of the Sun. Surface modes propagate in the convection zone, the depth of which depends on the solar helium abundance. If the helium abundance is increased, the central temperature rises to maintain the same central pressure and results in a steeper temperature gradient and convection zone. The observed oscillations can be fit with $Y=0.25 (\pm 0.01)$, the principal uncertainty arising from the equation of state of the Sun. However, the inferred central temperature is high enough to produce a solar neutrino flux that is well above experimental limits, which require $Y=0.19$. The resolution may well be that the neutrino possesses a slight mass. Even one-millionth of an electron volt would suffice to allow electron neutrinos to oscillate with muon and τ neutrinos between the Sun and the Earth, thereby removing the experimental discrepancy. Unfortunately, current experimental limits on the neutrino mass are only of order 1 eV.

Going outside the Solar System, M. Peimbert (Instituto de Astronomia, Mexico City) reported on the helium abundances in planetary nebulae, while P. Mezger (Max-Planck-Institut für Radioastronomie, Bonn) and P. Shaver (Garching) discussed Y_p determinations in HII regions. As HII regions are young (typically 1 Myr) and are expected to have acquired some helium from stellar enrichment of the interstellar gas, emission from and singly ionized helium is seen, and the principal uncertainty in evaluating Y is in estimating the amount of neutral helium. A well studied HII region, such as the Orion nebula, has $Y=0.29$, indicating very considerable enrichment of helium since the Solar System formed. However, attempts to infer the primordial helium abundance are much more controversial. By observing planetary nebulae and HII regions that display a range in metallicity, Z, Peimbert found a correlation between part of the helium abundance, ΔY , and the metallicity variations, ΔZ .

Attributing ΔY to the stellar enrichment that produced the observed heavy elements, the primordial helium abundance can then be inferred. Peimbert concluded that $Y_p=0.22 \pm 0.01$, but this low value depended critically on his finding that $\Delta Y/\Delta Z=3$.

The danger in this procedure was

demonstrated by D. Kunth (Institut d'Astrophysique, Paris), who studied several extragalactic HII regions. These were chosen because of their low metallicity, leading one to expect they should be less enriched by stellar nucleosynthesis than galactic HII regions. Kunth found no evidence for any correlation between helium abundance and metallicity. His conclusion was that $Y_p=0.25 (\pm 0.003)$ in the regions he studied, unless one regards this value as an upper limit since he could not rule out any stellar contribution to the observed helium.

With observers failing to establish definitively any $\Delta Y/\Delta Z$ correlation, crucial for interpolating to primordial Y , the theoreticians attempted to resolve the problem. It soon became apparent that theoretical models of the evolution of massive stars — prime candidates for substantial helium production — are in a similar state of disarray. The culprit here is the uncertain role of mass loss during stellar evolution. A. Maeder (Geneva Observatory) concluded that practically any correlation with $0 < \Delta Y/\Delta Z \leq 3$ was possible, when stellar models were combined with simple models of galactic evolution.

At this stage, the consensus on primordial helium was that with any value between 0.22 and 0.25 (or even 0.26) for Y_p being allowed by observation, practically any big-bang cosmology was possible. A very open or a closed universe was equally possible. However, helium is not the only element produced in the big bang, and other important elements are deuterium and lithium. New observations of the abundance of these elements were reported that promise to have a resounding impact on our understanding of galactic evolution, and indirectly constrain the amount of helium enrichment that is so crucial to determining Y_p .

Deuterium is partially destroyed by stars. The big-bang nucleosynthesis calculations predict a primordial deuterium abundance that is extremely sensitive to the mean baryonic density of the Universe. In a high-density or nearly closed universe, little deuterium survives the first few minutes. In addition, the stellar destruction (or astration) factor is unknown, and must be inferred from observations. The interstellar medium provides an environment where the current deuterium abundance can be measured. C. Laurent (Verrieres-le-Buisson) reported a re-analysis of deuterium absorption-line observations in galactic HI regions. One of the difficulties which plagued earlier analysis of these UV data obtained with the Copernicus satellite was the apparent variations along different lines of sight of the measured deuterium abundances relative to hydrogen. Laurent showed that small amounts of circumstellar hydrogen moving at high velocity were contributing to the apparent deuterium line profiles. In one instance, time variations of the line

*ESO Workshop on Primordial Helium, 2-3 February 1983, Garching bei München.

profiles confirmed this interpretation. Correction for this effect yielded an interstellar deuterium abundance of $5 (\pm 3) \times 10^{-6}$ by number relative to hydrogen.

D. Gautier (Observatoire de Paris, Meudon) presented results from the Voyager space probe studies of Jupiter and Saturn. Deuterated molecules were observed and, unlike the situation in interstellar molecular clouds, the temperature is believed to be too low for any appreciable fractionation to have occurred. The inferred presolar abundance of deuterium is $(2-5) \times 10^{-5}$ deuterium atoms per hydrogen atom. Since some five billion years of galactic evolution have elapsed between the formation of Jupiter and observed interstellar clouds, one infers an astration factor over this period of about 2, and perhaps as large as 8.

Such considerable astration exceeds that predicted in models of galactic evolution. Further confirmation that drastic revision of our understanding of galactic evolution is needed was provided by two observational coups from France. F. Spite (Observatoire de Paris, Meudon) presented his detections of lithium in old halo dwarf stars. The observed abundance of 10^{-10} lithium atoms per hydrogen atom does not depend on stellar temperature or metallicity, and so presumably reflects a more or less primordial value. As stressed by J. Audouze (Institut d'Astrophysique) in his introductory survey, such a low primordial abundance of lithium is practically impossible to reconcile with the revised interstellar deuterium abundance, unless very considerable galactic astration of deuterium is assumed. R. Ferlet (Verrieres-le-Buisson) announced the first detection of the lithium isotope ratio in the interstellar medium. Along one line of sight, he found that ${}^7\text{Li}/{}^6\text{Li} \approx 40$. Comparison with the Solar System value of 12.5 indicates that, since only ${}^7\text{Li}$ is produced in substantial amounts by the big bang, considerable stellar destruction or possibly synthesis of lithium must have occurred since formation of the Solar System. Finally, the interstellar abundance of the isotope ${}^3\text{He}$ was reported by T. Wilson (Max-Planck-Institut für Radioastronomie, Bonn), who detected the spin-flip radiofrequency transition of ${}^3\text{He}^+$ from three galactic HII regions and was able to measure directly the column density of ${}^3\text{He}^+$. He found that ${}^3\text{He}^+/\text{H}^+ = 5 \times 10^{-5} - 10^{-4}$ to within a factor of two for the ionized gas that he studied. The ionization correction to infer ${}^3\text{He}$ is unlikely to be large, and one may compare the result with the Solar System meteoritic abundance ${}^3\text{He}/\text{H} = 2 \times 10^{-5}$. Since ${}^3\text{He}$ is believed to be produced primarily by astration of deuterium, this result is again indicative of large deuterium astration over the past 5×10^9 yr.

The general consensus among astronomers was that the standard model of big-bang nucleosynthesis faced no in-

superable difficulties. Were new determinations of Y_p ultimately to restrict the model to the point where it was incompatible with the independently derived baryonic density, for example, there always remains the possibility of synthesizing much, if not all, of primordial helium in pregalactic stars. In an initially cold or tepid big bang, or in a highly anisotropic initial big bang, no helium need be produced. Pregalactic synthesis of helium by massive stars may also lead to a model which can naturally account for the other light elements. J. Audouze and J. Silk (Institut d'Astrophysique, CNRS, Paris) noted that in the absence of any pre-existing helium, cosmic-ray helium nuclei accelerated by winds from massive initially pure hydrogen stars would directly produce ample deuterium by spallation on ambient hydrogen without overproducing lithium or other heavy elements.

The principle excitement, however, lay not in such heretical speculations, but rather in the growing realization that galactic evolution models need severe revision. The apparent increases in abundance of both helium isotopes, as well as the decreases in deuterium and ${}^6\text{Li}$, since Solar System formation all point to very considerable astration over the past five billion years. The challenge is to theoreticians to develop models of galactic evolution that are capable of satisfying these constraints. As for observers, the value of the primordial helium abundance remains as elusive as ever, and much work has still to be done to provide values of Y_p that are capable of effectively constraining big-bang cosmology. □

Joseph Silk is on sabbatical leave at the Institut d'Astrophysique, Centre National de la Recherche Scientifique, 98 bis, Bd Arago, 75014 Paris from the University of California, Berkeley.

Protein microstructure

Why the eye lens is transparent

from George Benedek

THE article by M. Delaye and A. Tardieu¹ appearing in this issue of *Nature* (p.415) describes a beautifully balanced combination of experiment and theoretical analysis which convincingly describes the spatial distribution of protein molecules responsible for the transparency of the normal lens of the eye. In so doing, the authors have forged a vital link in the chain of reasoning needed to understand the microstructural changes in the positions of proteins responsible for cataract disease.

It has long been appreciated that some sort of correlation in the relative positions of the individual proteins making up the cytoplasm of the lens cells is necessary for transparency. Indeed, if the position of each protein were independently determined, then the total intensity of light scattered by a protein solution as dense as the lens cell cytoplasm would be sufficient to make the lens opaque. To overcome this difficulty Trokel² proposed in 1962 that the lens proteins were arranged in a very well ordered structure — a paracrystalline state — in which the relative position of proteins is precisely regular over very large distances. The diffraction of light by such ordered arrays will produce destructive interference between all the individual scattered wavelets, so the paracrystalline array would be completely transparent.

The new work by Delaye and Tardieu¹ demonstrates quite conclusively that no such paracrystalline state actually exists in the normal lens. In fact, their paper shows clearly that paracrystalline order is unnecessary to account for the observed

clarity of the lens.

Conceptually and historically the problem of the transparency of the lens is closely connected with the origin of transparency of the cornea. This tissue is made up of collagen fibres distributed in a mucopolysaccharide matrix having an index of refraction different from the collagen. Already in 1957, before Trokel's work on the lens, D. Maurice³ had pointed out that the density of collagen fibres in the cornea was so high that the cornea would be opaque if the fibre positions were uncorrelated. In response to this difficulty, he proposed in 1957 that a perfect lattice of collagen fibres existed in the cornea and destructive interference between scattered light wavelets ensured transparency of the medium.

Thus in both the cornea and the lens the prevailing concept in the 1960s, and one still taught in some medical schools, is that a long-range ordered lattice structure, be it of collagen or proteins, is necessary to produce a transparent cornea or lens.

The lattice theory of transparency of the cornea fell in the late 1960s and early 1970s. In that period a series of experimental and theoretical papers⁴⁻⁶ showed conclusively that only a limited degree of order was needed to account for the observed transparency of the cornea. In the cornea, order was achieved by a correlation in relative position of collagen fibres which extend only over a distance of three, or at the most four, neighbouring fibres. Only short-range order was needed theoretically, and only short-range order was found experimentally.

Following these discoveries it was assumed that the transparency of the lens was also achieved by a limited short-range

George Benedek is Professor of Physics at the University of California, Santa Barbara, California 93106.

order in the position of lens proteins. The paper by Delaye and Tardieu¹ demonstrates quite convincingly that this is the case. They establish the precise degree of short-range order that exists in the actual lens cytoplasm, and also in a series of protein solutions of varying density.

Using the techniques of small-angle X-ray scattering and light scattering they are able to establish a particularly striking result which is shown in their Fig. 3 (see p. 417). There they plot a measure of the turbidity of lens protein solutions as a function of the protein concentration. At first, there is the expected dramatic increase in turbidity of the solutions as the concentration is increased. But, when the density increases to the point that short-range inter-protein repulsion begins to produce a correlation in the position of near-neighbour

proteins, the turbidity actually begins to decrease with increasing protein concentration. Beyond this point the dense protein solutions grow ever more transparent as the concentration increases. The authors show that these results can be explained quantitatively by a hard sphere model for the extent of the correlations.

An additional gratifying result consists in a determination of the size of the constituent lens proteins from the measured hard sphere correlation distance. The size is in excellent agreement with independent biochemical determinations. □

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Plant sciences

Alien duckweed hiding quietly beside the Cam

from Peter D. Moore

It is difficult to be sure just how long it has been floating around on our waterways, but, apparently, we in Britain have an alien species of duckweed in our midst which is well established and, perhaps, fairly widespread. The discovery of the duckweed does not, however, bring credit to British botanists, for it was first found in a ditch beside the Cam, close to the centre of Cambridge, by a visiting German botanist, Professor E. Landolt¹. Now, *Lemna minuscula* (alias *L. minima*) has been the subject of a survey by Leslie and Walters² and has been recorded from a total of 23 localities, mainly in south-east England, and would seem to be holding its own alongside three native species of *Lemna*.

In days when additions to our established flora are very rare events³, botanists in Britain are paying increasing attention to casual aliens which are often brought into the country with transported goods and which rarely persist in the wild. As with the classic studies by Wilson and Taylor of ants in Polynesia⁴, one finds a very high rate of extinction among immigrant plants and apparent persistence is frequently due to replenishment of stocks from outside. As in the case of Wilson and Taylor's ants, however, certain immigrant, or 'tramp', species have secured a foothold, but mainly where competition from native species is low because of high levels of disturbance. A good example is the roadside weed thanet cress (*Cardaria draba*) which first came to these islands in 1809 from the Netherlands as a result of the dispersal on the Isle of Thanet, Kent, of hay from the

mattresses of sick soldiers returning from the Napoleonic Wars⁵. From Kent it has spread through much of southern England as a weed of disturbed roadsides and is now a common and well established alien in the chalk areas^{6,7}. A similar success story is attached to the speedwell *Veronica filiformis*, which spread from cultivation in rockeries during the 1920s.

Most of the aliens which have become well entrenched in our flora can be regarded as 'weed' species in the sense that they are competition sensitive and survive only in low-stress high-disturbance environments (*sensu* Grime⁸), but some have proved sufficiently aggressive and competitive to occupy niches in what appeared to be saturated habitats. One such species is the Canadian pondweed, a well known North American aquatic which was first recorded in Ireland in 1936, in Scotland in 1942 and in the midlands of England in 1947. The success of that invasive species has been attributed to its aptitude for vegetative propagation by fragmentation and its arrival at a time when canal transport was at its height. It is possible that the new duckweed will now repeat the success of its North American forerunner.

Lemna minuscula is very like the common *Lemna minor* in that the bulk of the plant is a flat, floating, modified stem with a single root. It differs in that it possesses a single short central nerve in place of the three nerves of *L. minor*. According to Leslie and Walters, this provides a reliable basis for identification but it can prove difficult to discern in the field; clearing by blocking in lactophenol may be necessary. It is entirely possible, therefore, that it has been, and is still being, overlooked.

The arrival of a new duckweed in Europe

(it has now also been found in France, Germany and Switzerland) raises the interesting question of whether it was transported across the Atlantic by migrating birds. Like the Canadian pondweed, it is capable of profuse vegetative spread, though in this case by budding, and the transport of some small fronds (say 0.5 mm in diameter) in mud on feather or feet, could easily result in wide-scale invasion. British ornithological reports contain ample evidence of regular wildfowl movements across the North Atlantic, particularly on the part of American wigeon (*Anas americana*), teal (*Anas crecca carolinensis*) and ring-necked duck (*Aythya collaris*)⁹.

There are, however, three difficulties with this idea. First, the majority of sightings of American wildfowl and other Nearctic vagrant birds occur in the west of the British Isles, whereas the occurrence of *Lemna minuscula* (with the exception of one site in Clwyd, North Wales) is restricted to southeastern Britain. It is possible, however, that this reflects the density of botanical field observers. Second, the distribution of *L. minuscula* in North America is essentially southern, from Georgia and Florida to California^{10,11}; there are no records for the north-east and eastern Canada^{12,13}, which is a more likely source area for errant wildfowl reaching Britain. Third, if birds were responsible for transatlantic transport of *Lemna* species, then *L. valdiviana* and *L. perpusilla* would be more likely candidates for carriage than *L. minuscula*, for their distribution extends into New England¹⁰. (It is interesting to note that a population of *L. minuscula* from Biarritz, south-west France, was determined as *L. valdiviana* when first discovered and is represented as such in *Flora Europaea*¹⁴.)

An alternative explanation of its advent, which is proposed by Leslie and Walters, is that it has been imported with aquatic plants and has spread from nurseries. Whatever its mode of arrival, it now remains to be seen whether it is sufficiently vigorous in our climatic conditions and in competition with species of *Lemna*, *Spirodela* and *Azolla* to maintain its newly found position in the British flora. □

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Peter D. Moore is a lecturer in the Department of Plant Sciences, King's College, 68 Half Moon Lane, London SE24 9JF.

Narrowband electromagnetic emissions from Jupiter's magnetosphere

D. A. Gurnett*, W. S. Kurth* & F. L. Scarf†

* Department of Physics and Astronomy, The University of Iowa, Iowa City, Iowa 52242, USA

† TRW Defense and Space Systems, One Space Park, Redondo Beach, California 90278, USA

Recent studies of wideband plasma wave data from Voyagers 1 and 2 have revealed the existence of narrowband radio emissions escaping from Jupiter's magnetosphere in the frequency range 1–12 kHz. These narrowband emissions are very similar to narrowband emissions previously discovered near Earth and Saturn, and are believed to be produced by mode conversion from locally generated upper hybrid resonance waves at odd half-integral harmonics of the electron cyclotron frequency. This mode conversion process is believed to be one of the basic mechanisms for generating planetary radio emissions.

DURING the Voyager 1 and 2 flybys of Jupiter, the plasma wave instrument detected a series of narrowband electromagnetic emissions at frequencies between ~1 and 20 kHz, in a frequency range where the radiation can propagate freely away from the planet. These emissions are remarkable because the spectral structure is extremely complex, consisting of many closely spaced lines, and because similar types of narrowband electromagnetic emissions are known to be generated in the magnetospheres of Earth¹ and Saturn^{2,3}. The frequency spacing of these radio emissions suggests that they are produced by electrostatic upper hybrid resonance waves at odd half-integral harmonics of the electron cyclotron frequency. This type of locally generated electrostatic wave is believed to be responsible for the narrowband emissions observed at Earth¹ using a mode conversion process. The mechanism for generating the narrowband electromagnetic emissions therefore appears to be a universal one, with possible applications to solar and other astrophysical radio sources. We now describe the characteristics of the jovian narrowband emissions and discuss possible mechanisms for generating the emissions.

Observations

An example of the jovian narrowband electromagnetic emissions is illustrated in Fig. 1 which shows a survey plot of the electric field intensities obtained from the 16-channel spectrum analyser during the inbound Voyager 1 pass through the

jovian magnetosphere on 3 March 1979. The narrowband emissions show up in these plots as a relatively smooth feature in the 10.0-kHz channel from about 08 h to 10 h SCET (spacecraft event time) at a radial distance of about 42 R_J . The narrowband character of the radiation can only be discerned by the use of high resolution spectrograms from the wideband electric field waveform data, such as shown in Fig. 2. These spectrograms, which consist of a sequence of 15-s intervals spaced a few minutes apart from about 9 h 40 min to 10 h 8 min, show that the radiation consists of a series of closed spaced narrowband emissions, each with a bandwidth of only a few hundred Hz, or less. These narrowband features are known to be freely propagating electromagnetic radiation because they occur well above the electron plasma frequency, f_p , in a frequency range where the only mode of propagation is the free space electromagnetic mode. The electron plasma frequency can be determined from the trapped continuum radiation, which is known from wideband measurements to have a low frequency cutoff at the local electron plasma frequency⁴. The electron cyclotron frequency, f_c , which is the only other relevant frequency of the plasma is ~400 Hz in this region, well below the electron plasma frequency.

The sequence of spectrograms in Fig. 2 shows that although the narrowband emissions do not change significantly during the 15-s duration of the spectrogram, marked changes occur during the several minute interval between spectrograms.

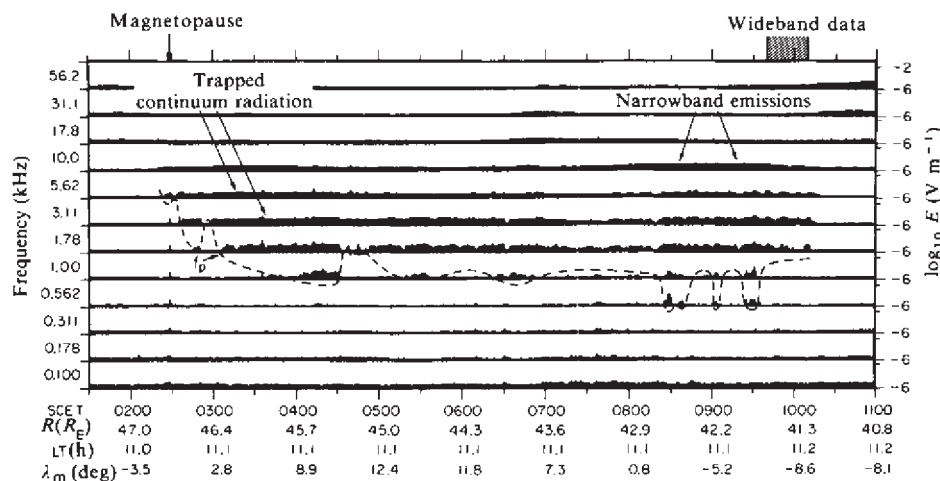
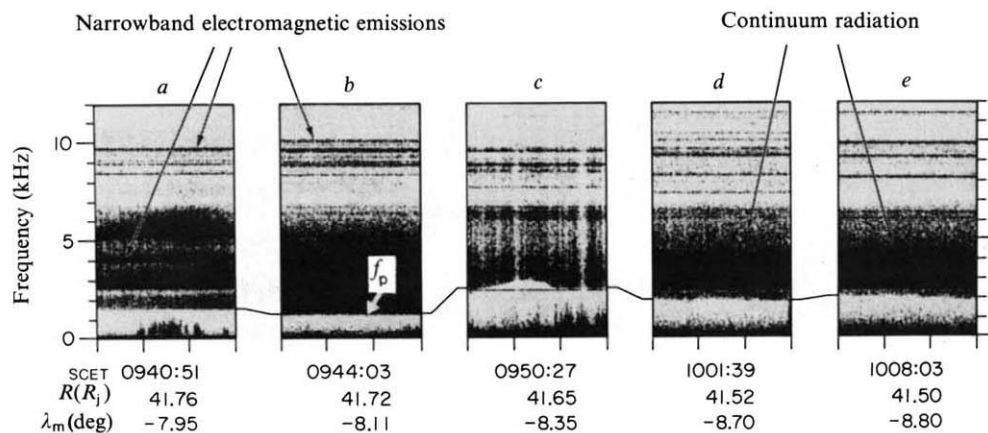


Fig. 1 The electric field intensities from the 16-channel spectrum analyser on Voyager 1 during the inbound pass through the jovian magnetosphere 3 March 1979. The narrow-band electromagnetic emissions occur in the 10-kHz channel. The dashed line indicates the electron plasma frequency, $f_p = 9\sqrt{n_e}$ kHz, where n_e is the electron number density in cm^{-3} .

Fig. 2 High-resolution wideband spectrograms showing the 10-kHz emission in Fig. 1 consists of many closely-spaced narrowband emissions. The broadband emission just above the plasma frequency, f_p , is continuum radiation trapped in the low density magnetospheric cavity.



Unfortunately, because gaps occur in the wideband data⁵, it is not possible to follow the detailed temporal evolution. However, the spectrograms give the strong impression that the source consists of a large number of isolated lines between about 8 and 12 kHz, each of which is changing intensity on a time scale of a few minutes. The electric field strengths of the most intense bands range from about 10 to 30 $\mu\text{V m}^{-1}$. Occasionally, as in Fig. 2d at 7.4, 8.3 and 9.2 kHz, a series of lines can be identified that have a nearly constant frequency spacing, suggesting that the emission frequencies are harmonically related. In addition to the lines around 10 kHz another series of lines can be seen at lower frequencies in Fig. 2a superimposed on the trapped continuum radiation. These lower frequency emissions, which are shown in greater detail in Fig. 3 at 2.7, 4.1 and 5.4 kHz, also have a harmonic frequency spacing. Similar narrowband features have been previously reported in the trapped continuum radiation at Jupiter⁶. The lines in the trapped continuum radiation are usually not as narrow and sharply defined as the emissions observed above the continuum radiation.

At present, three events have been identified near Jupiter with characteristics of the type described above. The second of these events occurred during the outbound Voyager 1 pass through the magnetotail on 6–7 March 1979, at a radial distance of about 32 R_J . The spectrum analyser data for this event are shown in Fig. 4. The narrowband emissions in this case sweep downward in frequency, starting in the 17.8-kHz channel at about 22 h on 6 March and ending in the 5.62-kHz channel at about 3 h on 7 March. A sequence of spectrograms illustrating the narrowband character of these emissions is shown in Fig. 5. In this case the spectrograms have a 4-s duration and are separated by roughly half-hour intervals. Again the spectrum is characterized by many closely spaced lines with a tendency for harmonic frequency spacings. The downward drift in the average frequency is clear. The temporal variations of the individual lines are very complex. Some of the lines decrease in frequency with increasing time, whereas others remain at a fixed frequency, varying in intensity as the main band sweeps across the emission frequency. The downward frequency drift continues until the lines merge into the trapped continuum radiation below ~ 5 kHz.

The third event was obtained during the Voyager 2 approach to Jupiter on 4 July 1979, at a radial distance of 71.5 R_J , very close to magnetopause. This case, shown in Fig. 6, has very clear evidence of harmonic structure with a frequency spacing of about 200 Hz. Also, evident in the same region is a series of narrowband upper hybrid resonance (UHR) waves. From previous observations⁶ these waves have been identified as an electrostatic mode at the local upper hybrid resonance frequency, $f_{\text{UHR}} = (f_p^2 + f_c^2)^{1/2}$. The upper hybrid waves occur at discrete frequencies separated by the electron cyclotron frequency. Figure 6 shows that the upper hybrid waves occur at the same frequency as the narrowband electromagnetic

emissions, which strongly implies that the escaping electromagnetic radiation is being generated by these locally generated electrostatic waves.

Because the jovian decametric radiation⁷ and kilometric radiation^{6,8}, are controlled by the rotation of Jupiter and tend to occur in a fixed longitude range, it is interesting to investigate the longitude at which the narrowband emissions occur. For the events on 3 March and 6–7 March the System III (1965) longitude of the spacecraft was $332^\circ \pm 36^\circ$ and $4^\circ \pm 105^\circ$, respectively. For the event on 4 July the System III (1965) longitude was 314° . No longitude limits can be given for the third event because the duration cannot be accurately determined from the 16-channel survey data. These longitudes all fall in the same general region, centred on roughly 336° , suggesting that the rotation of Jupiter may have some control on the occurrence of these emissions. However, with only three events available for study, it is difficult to determine the strength of this rotational control.

Discussion

These observations of jovian narrowband electromagnetic emissions confirm the existence of the same type of radio emission process at three planets: Earth, Jupiter and Saturn. The similarity between the jovian and saturnian narrowband emissions is quite striking. At Saturn the wideband spectrograms show a series of narrowband emission lines^{2,3} with characteristics essentially identical to those illustrated in Figs 2, 3 and 5. At Earth wideband spectrum measurements with the ISEE 1 spacecraft show essentially identical narrowband

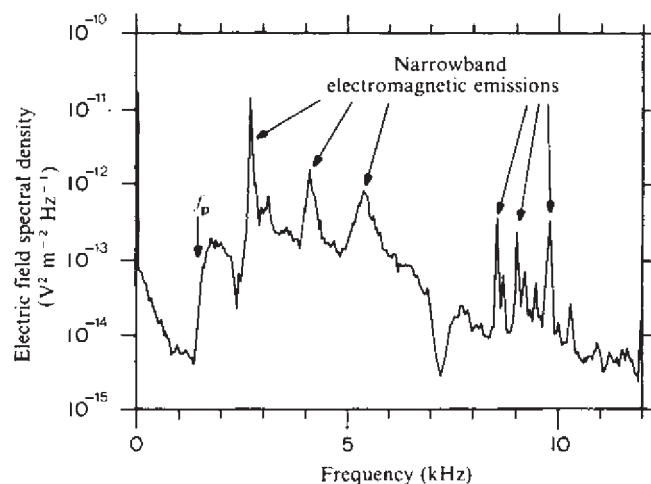


Fig. 3 A representative electric field spectrum for the event in Fig. 2a at 0940:48 SCET; 18-s average; $R = 41.7 R_J$; $\lambda_m = -8.1^\circ$; LT = 11.1 h. This spectrum is remarkably similar to the spectrum of the narrowband electromagnetic emissions observed at Saturn³.

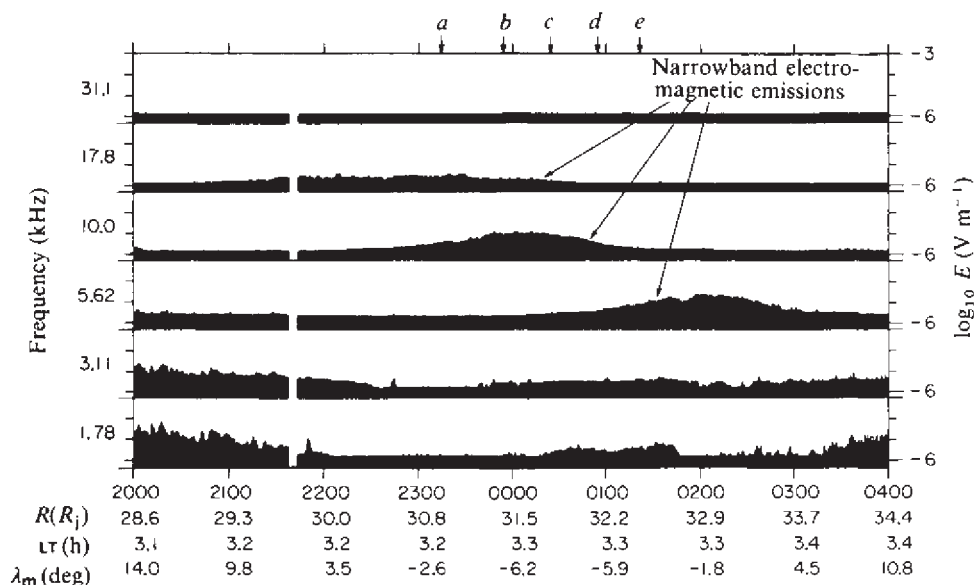


Fig. 4 The 16-channel spectrum analyser data from Voyager 1 during the outbound pass through the magnetotail 6–7 March 1979. The narrowband emissions occur in the 17.8-, 10.0- and 5.62-kHz channels, sweeping downward in frequency with increasing time.

emission lines (see Fig. 7 of ref. 1). The jovian narrowband emissions may also be similar to a type of jovian radio emission called narrowband kilometric radiation (nKOM) that has been previously reported by Kaiser and Desch⁹ from the planetary radio astronomy (PRA) instrument on Voyager. The nKOM usually consisted of an enhancement in a few channels near 100 kHz, above the frequency range (1–12 kHz) of the emissions described here. It seems likely that if better frequency resolution were available from the PRA instrument it would be found that the nKOM actually consists of many closely spaced emission lines similar to those illustrated in Fig. 3.

The best evidence of the origin of the narrowband electromagnetic emissions comes from terrestrial measurements, where a long series of observations indicates that the radiation is produced by electrostatic electron cyclotron waves near the upper hybrid resonance frequency^{10–12}. The upper hybrid resonance instability occurs when the upper hybrid resonance frequency is near an odd half-integral harmonic of the electron cyclotron frequency, $f_{UHR} = (n + 1/2)f_c$, where n is an integer^{12–15}. The free energy for the instability is produced by a region of positive slope, $\partial f/\partial v > 0$, in the electron distribution function, such as occurs in a ring or loss-cone distribution. The instability is enhanced by the presence of cold electrons.

The physical situation that is believed to be responsible for generating the narrowband emissions in the jovian magnetosphere is illustrated in Fig. 7, which shows a representative radial profile of the upper hybrid resonance frequency and the electron cyclotron frequency near the equatorial plane. Because the plasma is moderately dense near the equatorial plane the upper hybrid resonance is nearly the same as the electron plasma frequency, $f_{UHR} \approx f_p$, and is therefore determined by the electron density profile. The dashed lines are the odd half-integral harmonics of the electron cyclotron frequency. The electrostatic waves occur at the points where the dashed lines cross the upper hybrid resonance frequency. Observations in the Earth's magnetosphere show that the electrostatic waves are converted directly to electromagnetic radiation with little or no frequency shift¹. The mode conversion process could be either a linear escape mechanism associated with density gradients, of the type suggested by Oya¹⁶ and Jones¹¹, or a nonlinear two-wave interaction, of the type discussed by Melrose¹⁷. Because of the quantized frequency spectrum of the upper hybrid waves, the escaping radio emissions consist of a series of lines with a quasiharmonic frequency spacing, the exact frequencies of which are determined by the details of the electron density and magnetic field profiles. Because the electron density at Jupiter is often very irregular (note the variations of f_p in Figs 2 and 5), the emission frequencies are undoubtedly

much more complicated than indicated by Fig. 7, thereby accounting for the large number of lines and great complexity of the spectrum. Observations in the Earth's magnetosphere indicate that the source of the narrowband emissions is very patchy¹, consisting of numerous isolated regions, each emitting a frequency determined by the plasma parameters associated with that region.

As indicated in Fig. 7 the narrowband emissions can, in principle, be generated either on the rising part of the electron density profile in the inner magnetosphere or in the region of rapidly decreasing density near the magnetopause. The line spacing of the emissions in Fig. 6 (~200 Hz) is characteristic of the electron cyclotron frequency in the outer region of the magnetosphere, which suggests that these emissions are generated at or near the magnetopause. The radiation is then emitted inward, towards the planet, as shown in Fig. 7. In other cases, such as in Figs 2 and 5, the emission frequency extends to frequencies so high, well above 10 kHz, that a magnetopause source is impossible. In these cases the radiation is emitted outward, from a source in the inner magnetosphere. If the emission frequency is below the magnetopause plasma frequency, then the radiation is permanently trapped inside the low density magnetospheric cavity. This radiation can then account for the trapped continuum radiation, as has been

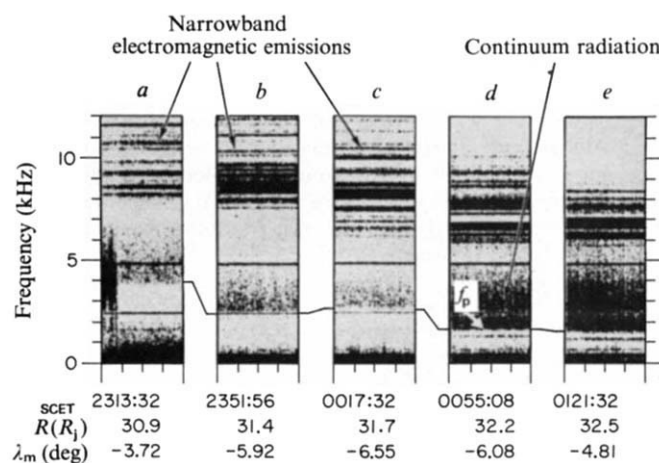


Fig. 5 A sequence of high resolution spectrograms for the event shown in Fig. 4. Note that downward frequency drift with increasing time. This downward drift is suggestive of a source moving outward from Jupiter, emitting near the local electron plasma frequency as the source moves to increasing radial distance.

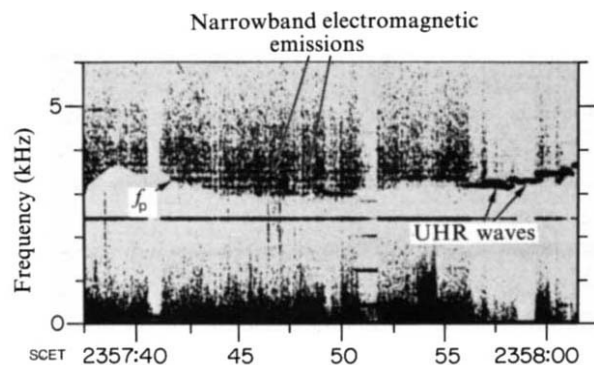


Fig. 6 A high-resolution spectrogram of narrowband electromagnetic emissions detected near the magnetopause during the Voyager 2 encounter 4 July 1979; $R = 71.5 R_J$; $\lambda_m = 5.6^\circ$; $LT = 10.3$ h. The UHR waves are electrostatic emissions generated near the upper hybrid resonance frequency, f_{UHR} . These emissions occur at discrete frequencies separated by the electron cyclotron frequency, $f_c = 28B$ Hz, where B is the magnetic field in nT.

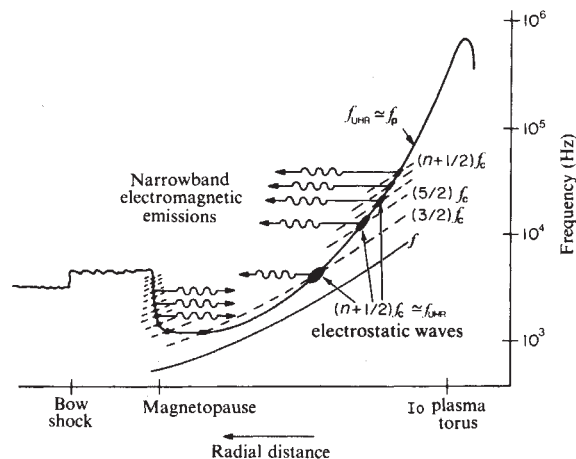


Fig. 7 A model illustrating a mechanism for generating the narrowband electromagnetic emissions in the jovian magnetosphere. An electron cyclotron instability is believed to produce electrostatic waves at $(n+1/2)f_c \approx f_{UHR}$. These waves are then converted to electromagnetic radiation by a mode coupling process.

previously suggested^{18,19}. The broadened width of the lines detected in the trapped continuum radiation (see Fig. 3) is believed to be due to fluctuations in the position of the magnetopause, which cause Doppler broadening as suggested by Barbosa¹⁹. If the emission frequency is above the magnetopause plasma frequency, then the radiation can propagate freely away from the planet.

The downward frequency drift for the event shown in Fig. 5 is strongly suggestive of a source moving outward away from the planet, emitting radiation near the local electron plasma frequency, similar to the mechanisms involved in Type II and Type III solar radio bursts. Based on the jovian electron density profile given by Gurnett *et al.*²⁰, the narrowband emissions in Fig. 5 would then be generated at radial distances of ~ 15 – $25 R_J$ in the outer regions of the Io plasma torus. From the observed frequency drift rate the radial velocity of the source is estimated to be about $3R_J h^{-1}$ or 60 km s^{-1} . It is unlikely that the source motion could be produced directly by charged particles moving outward along the magnetic field lines comparable with Type III solar radio bursts, because the energies, 0.01 eV for an electron and 18 eV for a proton, are too small to produce plasma instabilities in the hot jovian plasma. More likely the source is associated with a cloud of plasma moving outwards through the magnetosphere near the equatorial plane. Interchange instabilities caused by the centrifugal force in the rapidly rotating magnetosphere of Jupiter are expected to cause radial transport of plasma outward from the Io plasma torus, perhaps producing isolated clouds of plasma moving outward through the magnetosphere. Isolated events of the type observed could be produced by temporal variations in the plasma source, possibly caused by changes in the volcanic activity of Io, which is the primary source of plasma in the jovian magnetosphere. Because the upper hybrid resonance instability is sensitive to the cold to hot plasma density ratio^{12–15,21}, a cloud of relatively

cool plasma from the Io torus could trigger the upper hybrid resonance instability as it moved through the hot outer regions of the magnetosphere.

It is also possible that the radio emission could be associated with a transient cloud of plasma that is decaying in density at a fixed radial distance. In principle, a stationary decaying cloud can be distinguished from a moving cloud, because for a stationary cloud the frequency spacing, which is controlled by the magnetic field, should remain constant, whereas for a cloud moving radially outward the frequency spacing should decrease with increasing radial distance. In practice, because of the limited amount of wideband data available and the considerable complexity of the spectrum we are not able to distinguish these two possible interpretations.

Finally, we consider the possibility that the frequency drift could be caused by a frequency dependent latitudinal beaming of the radiation such as proposed by Jones²² for the jovian kilometric radiation. In this case the frequency shift would be caused by the changing magnetic latitude of the spacecraft as the planet rotates. This beaming mechanism predicts both upwards and downwards frequency drifts depending on the phase of the planetary rotation. At present, no events with an upward frequency drift have been observed. Furthermore, according to Jones the frequency should increase with increasing latitude, opposite to the latitudinal variation evident for the event in Fig. 5. Thus, the latitudinal beaming model does not appear to provide a likely explanation for the observed frequency drift.

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Mechanism of ribosome frameshifting during translation of the genetic code

R. Weiss & J. Gallant

Department of Genetics SK-50, University of Washington, Seattle, Washington 98195, USA

*Some frameshift mutations are strongly suppressed by limitation for particular aminoacyl-tRNA species. Here, we show that ribosome frameshifting at a specific tryptophan codon during Trp-tRNA limitation accounts for suppression of a group of downstream frameshift alleles in the *rII*B gene of bacteriophage T4. Genetic and physiological observations strongly suggest that ribosome frameshifting at this position depends on the binding of a noncognate (leucine) tRNA.*

TRIPLET reading of the genetic code is the hallmark of protein synthesis. Nonetheless, most frameshift mutations exhibit detectable residual activity at a level of roughly 10^{-4} – 10^{-3} (ref. 1). This residual activity demonstrates that ribosomes occasionally shift spontaneously into an alternative reading frame, thus compensating for or suppressing the effect of a frameshift mutant allele. In principle, such compensatory frameshifts might occur either at the mutant position itself, or at a nearby position.

Limitation for any one aminoacyl-tRNA species favours mis-sense amino acid substitutions at codons calling for the species in short supply, especially in *Escherichia coli* *rel* mutants deficient in the production of ppGpp (refs 2–6). We have previously noted that the same conditions increase the frequency of spontaneous ribosome frameshifting, indicated by phenotypic suppression of a subset of *lacZ* frameshift mutations⁷. The pattern observed was specific with regard to both frameshift allele and aminoacyl-tRNA species: that is, each frameshift allele which exhibited suppression did so only during limitation for a particular small group of amino acids, differing from one suppressible allele to another.

Our tentative interpretation^{7–9} was that specific codon positions in mRNA were prone to ribosome frameshifting when translation of these positions was slowed through a scarcity of the cognate aminoacyl-tRNA. We postulated that the specificities of phenotypic suppression reflected the distribution of 'shifty' codon positions sufficiently close to each suppressible allele, a phenotypic analogue of the classic Cambridge studies of mutual genetic suppression by frameshift alleles of opposite sign in the *rII*B gene of bacteriophage T4 (refs 10, 11).

We have now used the *rII*B system to test these ideas. Pribnow and colleagues have recently established the nucleotide sequence of the relevant portion of the *rII*B gene¹²; the detailed genetic data of the Cambridge group¹¹ permit the mapping of each frameshift allele onto the nucleotide sequence to an accuracy of a few base pairs¹². With this background of sequence information in mind, we have sought to locate specific sites of ribosome frameshifting during aminoacyl-tRNA limitation, and to inquire into the mechanism of frameshifting at one site we have identified.

A site of phenotypic frameshift suppression

We have tested a large number of *rII*B frameshift mutants for phenotypic suppression during limitation for a variety of amino acids, a complete account of which will be reported elsewhere (R.W. and J.G., manuscript in preparation). Here, we will concentrate on the strongest cases of phenotypic suppression we encountered in this survey. A block of (+) frameshift alleles located between nucleotides 100 and 131 of the message exhibit a huge increase in phage transmission when the restrictive host cells are subjected to partial inhibition by indoleacrylic acid (IA), a tryptophan antagonist, during the first 30 min of the lytic cycle. (Wild-type phage development resumes after reversal of tryptophan limitation, so that final phage yield is

little affected (ref. 13 and our observations).) Figure 1 summarizes these results, with the relevant frameshift alleles positioned on the messenger nucleotide sequence according to the data of Pribnow *et al.*¹². Table 1 presents our experimental results in fuller detail. Phenotypic suppression was abolished by the provision of tryptophan, and unaffected by the provision of the other 19 amino acids; moreover, phenotypic suppression was also elicited by inactivation of a temperature-sensitive tryptophanyl-tRNA synthetase (Table 1b). The effect is therefore unquestionably due to some consequence of a limited supply of Trp-tRNA.

The suppression can be explained by a compensating phenotypic shift from the normal to the (–) reading frame somewhere between FC36⁺, which is not suppressed, and FC54⁺, which is. On this view, suppression could not extend beyond the minute barrier (*m*^b in Fig. 1), a (–) frame UGA terminator codon just beyond FC38⁺. In agreement, we find that 370⁺, the first (+) frameshift allele downstream of the minute barrier¹², is not suppressed.

The short stretch of the *rII*B message between FC36⁺ and FC54⁺, only a few codons long, contains the sole in-frame tryptophan (UGG) codon in the first third of the message. If the compensating frameshift evoked by Trp-tRNA limitation occurs at this UGG position, alteration of this codon should abolish the effect. HB74am, which is synonymous with X655 UAG, is a UAG mutant allele of this position. In the presence of the amber suppressor *supF* in the host cells, this position is generally read by the suppressing Tyr-tRNA, and is relatively unaffected by Trp-tRNA limitation (Table 1c, line

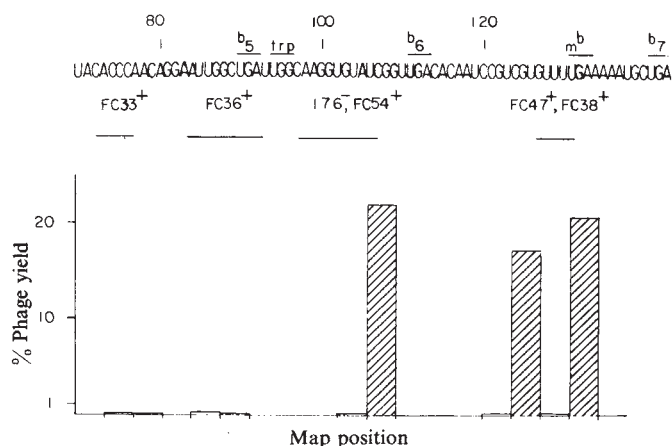


Fig. 1 Relevant area of the *rII*B genetic and physical maps; for more detail see ref. 12. Numbering is from the first nucleotide of the *rII*B AUG start codon. The level of phenotypic suppression during tryptophan limitation is aligned with the map position of the (+) frameshift mutations depicted: open bar, normal infection; hatched bar, tryptophan-limited infection. See Table 1 for details.

5). In such host cells, translation of the double mutant HB74am·FC38⁺ differs from the normal situation only in that the wild-type tryptophan codon upstream of FC38⁺ is now in effect a tyrosine codon. Table 1c shows that this alteration abolishes phenotypic suppression of FC38⁺ by Trp-tRNA limitation.

Two conclusions follow from this experiment. First, the compensating frameshift which suppresses FC38⁺ must occur at the tryptophan position in question, nucleotides 94–96 of the message. Second, phenotypic frameshifting at this position depends on the absence of aminoacyl-tRNA cognate to that position.

The induced frameshift at this position shows directional specificity. A phenotypic shift into the (+) reading frame would suppress 176⁻, located in the same region as the responsive FC54⁺, yet we detect no suppression of this (-) frameshift allele.

Identification of the suppressed *rII*B protein

One alternative to frameshifting at positions 94–96 needs consideration. Napoli *et al.* have shown that the anomalously high residual activity of certain double-mutant combinations of two (+) frameshift alleles can be explained by reinitiation in the (-)

reading frame near the point of termination at the barrier following the first (+) frameshift mutation¹⁴. Their demonstration of this mechanism rests on identification of truncated *rII*B products, on SDS gel electrophorograms, with shortened lengths corresponding to the positions of potential reinitiation sites in the (-) reading frame¹⁴.

A similar mechanism might account for the present phenomenon, through premature termination at codons calling for a limiting aminoacyl-tRNA followed by out-of-phase reinitiation. Potential reinitiation codons in the (-) reading frame can be found upstream of FC54⁺. Were this the mechanism of phenotypic suppression by tryptophan limitation, the suppressed *rII*B protein would be at least 30 amino acids shorter than the normal full-length protein. In contrast, frameshifting at position 94–96 would yield a full-length *rII*B product. We have distinguished between these two mechanisms by sizing the suppressed *rII*B protein, following the procedure of Napoli *et al.* Figure 2 shows the results. The normal *rII*B protein is evident as a band of molecular weight 33,000 in cells infected by wild-type phage, and absent in cells infected by 638Δ, FC47⁺, 514⁺ (located between b₆ and FC47⁺) and FC36⁺·FC47⁺, a reinitiating double (++) combination¹⁴. It can be seen that tryptophan-limited suppression of FC47⁺ and 514⁺ generates a band at the position of full-length *rII*B protein. We conclude that suppression proceeds through frameshifting at positions 94–96, and not through termination and reinitiation.

We have detected one other type of phenotypic suppression which is almost as efficient as the tryptophan-limited suppression under consideration here. FC151⁻ is a (-) frameshift allele located in a run of four A residues (thus a lysine codon), and FC87⁻ is another (-) allele just downstream¹². Both mutants exhibit a 100-fold increase in transmission when Lys-tRNA synthetase is partially inhibited by lysine-hydroxamate. As Fig. 2c,d demonstrates, here again a full-length *rII*B protein can be detected. In this case, the phenotypic frameshift, presumably at the aforementioned lysine codon, must be into the (+) reading frame, so as to suppress (-) frameshift mutants. Thus, frameshifting in each direction may occur at different shifty positions.

The relative proportion of full-length product, determined by densitometry, is given in Fig. 2 legend. It can be seen that our indirect measure of suppression through phage yield is in good agreement with a direct measurement of the suppressed protein.

Mechanism of phenotypic frameshifting

Why does tryptophan limitation elicit a shift from the normal to the (-) reading frame at the UGG codon of positions 94–96? Two observations suggest that this shift is a consequence of incorrect tRNA recognition. First, the stringent response determined by the *relA*⁺ gene is known to be a general antagonist of incorrect tRNA binding^{2-7,15} apparently through the effect of ppGpp on elongation factor Tu (ref. 16). For this very reason, all the experiments we have discussed thus far were conducted with *relA*⁻ host cells. Table 2 shows that tryptophan-limited suppression of FC38⁺ is severely diminished in isogenic host cells which are *relA*⁺. Second, the *rpsL-A1* (formerly called *strA1*) allele of the gene coding for ribosome protein S12 is known to restrict incorrect tRNA recognition¹⁷. Table 2 shows that this mutant allele also severely restricts suppression of FC38⁺ by tryptophan limitation.

These results strongly suggest that incorrect tRNA binding at the UGG position in question is responsible for the phenotypic frameshift. Which tRNA species might be responsible for this? Reference to the genetic code shows that the following tRNAs might misread UGG by a one base error: tRNA^{Arg} and tRNA^{Gly} (first position error); tRNA^{Ser} and tRNA^{Leu} (second position error); and tRNA^{Cys} (third position error). We have sought the identity of the tRNA responsible by systematically limiting the acylation of each of these classes of tRNA, as well as three more distantly related tRNAs, simultaneously with tryptophan limitation.

Table 1 Tryptophanyl-tRNA limited suppression of *rII*B frameshift alleles

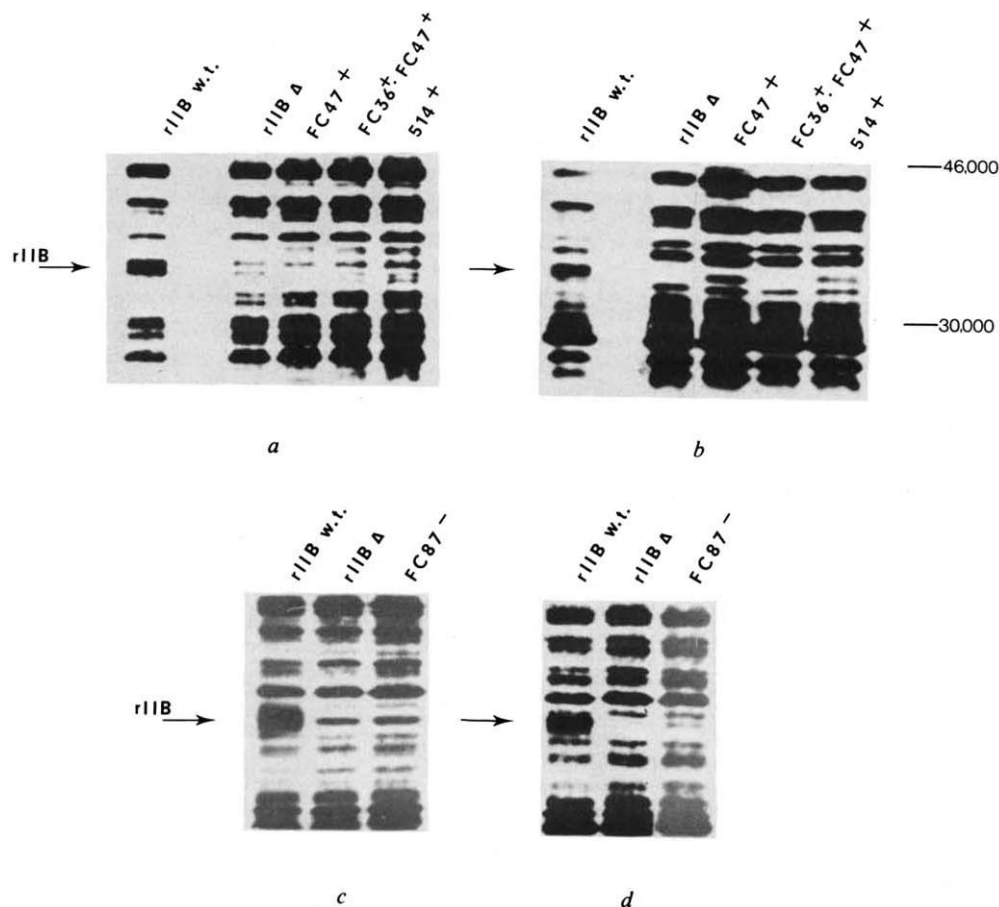
<i>rII</i> B allele	% Maximum phage yield (±s.e.m.)		
	Normal infection	Trp-limited	$\bar{x}$ -fold increase
638Δ	0.019 ± 0.001	0.023 ± 0.010	1.2
FC33 ⁺	0.008 ± 0.003	0.010 ± 0.008	1.3
FC36 ⁺	0.225 ± 0.027	0.124 ± 0.057	0.6
FC54 ⁺	0.048 ± 0.015	22.0 ± 2.3	458
FC47 ⁺	0.066 ± 0.022	17.3 ± 2.5	262
FC38 ⁺	0.065 ± 0.019	20.9 ± 4.0	322

<i>rII</i> B allele	30 °C	39 °C	$\bar{x}$ -fold increase
	% Maximum phage yield	% Maximum phage yield	
638Δ	0.023	0.029	1.3
FC36 ⁺	0.31	0.22	0.7
FC38 ⁺	0.59	4.9	8.3
FC47 ⁺	0.14	3.3	23.6
X655 UAG	2.4	2.9	1.2

<i>rII</i> B allele	Normal infection	Trp-limited	$\bar{x}$ -fold increase
	% Maximum phage yield	% Maximum phage yield	
638Δ	0.0038	0.0039	1.0
176 ⁻	0.018	0.024	1.3
HB74am · FC38 ⁺	0.40	0.20	0.5
FC38 ⁺	0.028	13.5	483
X655 UAG	37.5	23.1	0.6

Phenotypic suppression of *rII*B mutations is measured by titrating, on a permissive *E. coli* strain, the phage produced after a single growth cycle in a restrictive host, a λ lysogen of *E. coli* K-12. The infections were done at a phage multiplicity of 0.1, on actively growing cells at a density of 2×10^8 cells ml⁻¹. The growth medium was M9 + the amino acid supplements required for growth; 6-methyl-D,L-tryptophan served as the cofactor for phage adsorption in the tryptophan-limited infections. Phage were added at $t = 0$ min, unadsorbed phage were titrated and removed with anti-T4 sera at $t = 12$ min, infectious centres were titrated and diluted to prevent re-adsorption at $t = 30$ min, and phage yield measured at $t = 120$ min, after CHCl₃ addition. The % phage yield is just the percentage of phage produced from the *rII*B mutation infection relative to the *rII*B wild-type infection. *a*, Trp-limitation is with $20 \mu\text{g ml}^{-1}$ 3β-indoleacrylic acid + $20 \mu\text{g ml}^{-1}$ 6-methyl-D,L-tryptophan. This level of inhibitor slows the host growth rate by 40%, and the effect of this starvation on phage production is negligible¹³. s.e.m. is the standard error of the mean for three replicate experiments. The host is Hfr H 3000, *relA*⁻, (Δc1857) at 30 °C. *b*, The host is Hfr H 3000, *relA*⁻, *trpS*^{ts} (Δ), for lines 1–4, and CP79 *relA*⁻, *trpS*^{ts} (Δ), *supE44* (=Gln-inserting amber suppressor) for line 5. *c*, The host is Hfr H 3000 *relA*⁻ (Δ) *supF66* (=Tyr-inserting amber suppressor). Trp-limitation is the same as in *a*.

Fig. 2 *E. coli* Hfr 3000 *relA*⁻ was grown at 30 °C in M9 minimal media, the cells were infected at a multiplicity of infection of 7 and 1 μ Ci ml⁻¹ ¹⁴C-leucine, alanine, arginine and glutamate was added 7–12 min post-infection. Infected cells were lysed in SDS sample buffer and loaded onto 10% SDS-acrylamide gels, pH 8.7. Gels were enhanced (NEN Enhance) and exposed at -70 °C. *a*, No aminoacyl-tRNA limitation; *b*, +20 μ g ml⁻¹ indoleacrylic acid + 20 μ g ml⁻¹ 6-methyl-D,L-tryptophan; *c*, No aminoacyl-tRNA limitation; *d*, +100 μ g ml⁻¹ lysine hydroxamate. Densitometric scanning revealed that the percentage of wild-type *rII*B product in Trp-limited FC47⁺ and 514⁺ infected cells was: 12.3% and 11.5%, respectively; and in the lysine-hydroxamate-treated FC87⁻ infected cells: 2.9%. w.t., wild type.



The results (Table 3) reveal a striking degree of specificity. Tryptophan limitation elicits strong phenotypic suppression of FC38⁺ whether or not the acylation of Gly, Cys, Ser, Arg, Thr, Val or His tRNA is limited at the same time. However, limitation for Leu effectively reverses the Trp-limited suppression of FC38⁺. Note that we have limited the acylation of tRNA^{Leu} in three different ways with the same result: starvation of a leucine auxotroph, inhibition of the first enzyme of the leucine pathway by azaleucine¹⁸ and inactivation of a temperature-sensitive leucyl-tRNA synthetase.

The double limitation regime involves certain ambiguities which must be adequately controlled. One is nonspecific inhibition of phage productivity due to severe inhibition of protein synthesis during the period of double amino acid limitation. We have monitored this effect by measuring the productivity of an amber-suppressed nonsense mutation at the site of the Trp codon at position 94–96, X655am, whose transmission is about the same as that brought about by tryptophan-limited phenotypic suppression of FC38⁺. Table 3a, column 3, shows that double amino acid limitation has relatively little effect on phage productivity. The effect of double amino acid limitation relative to the single Trp-limitation of FC38⁺ is normalized to the identical value for the amber-suppressed X655 in Table 3a, column 5. This controls for any effect other than frameshifting in the vicinity of the UGG codon at positions 94–96, and establishes a metric for the specific decrease in frameshifting at this position.

A second ambiguity is that limitation for a second amino acid might so reduce the demand for Trp-tRNA that the tryptophan limitation is ineffective in draining tRNA^{Trp} of its amino acid. We can rule out this possibility on two grounds. First, such a mechanism should not be restricted to leucine among all the second amino acids tested. Moreover, we have obtained an independent estimate of the activity of Trp-tRNA^{Trp} in protein synthesis in conditions of single and double amino acid limitation. UGA mutants are notoriously leaky, due to misreading by tRNA^{Trp} (refs 19, 20). Tryptophan limitation by indolea-

Table 2 The absence of suppression in *relA*⁺ and *rpsL* hosts for Trp-limited suppression

<i>rII</i> B allele	% Maximum phage yield			
	Normal infection	Trp limited	Fold-increase	
<i>a</i>				
FC38 ⁺	0.021	0.188	9.0	{ CP78 <i>relA</i> ⁺ ; compare with Table 1
FC33 ⁺	0.037	0.026	0.7	
N24UGA	38.5	1.2	0.03	
<i>b</i>				
638Δ	0.0049	0.0034	0.69	{ <i>strA</i> ; compare with Table 1
FC38 ⁺	0.0071	0.018	2.6	
FC47 ⁺	0.0021	0.0069	3.3	

a, The host is CP78 *relA*⁺ (λ) at 30 °C. Trp-limitation is with 20 μ g ml⁻¹ 3-indoleacrylic acid + 20 μ g ml⁻¹ 6-methyl-D,L-tryptophan. The infection procedure is the same as in Table 1. *b*, The host is Hfr H 3000 (λ cI857) *rpsL*1 at 30 °C, and Trp-limitation is the same as above.

rylic acid reduces the transmission of the UGA mutant N24UGA about 15-fold, as shown in Table 3b, reflecting the expected reduction in Trp-tRNA. As Table 3a shows, double limitation for leucine, threonine or histidine in addition to tryptophan fails to restore the UGA misreading activity of Trp-tRNA to any significant degree, proving that tRNA^{Trp} remains largely deacylated during double limitation for tryptophan and the second amino acid.

The double limitation experiments rule out, at least for this position, a mechanism proposed by Atkins *et al.*²¹. These workers showed that high levels of a tRNA^{Ser} which reads AGC stimulates frameshifting in the MS2 coat gene *in vitro*. They postulated that correct reading of an AGC codon in the wrong reading frame by this tRNA promoted the shift. In the present case, delay in translating the UGG codon at 94–96 might

Table 3 Specific reversal of Trp-limited suppression by double amino acid limitation

a, Level of suppression	= $\frac{\text{phage yield (Trp-limited and amino acid 2-limited)}}{\text{phage yield (Trp-limited)}}$			
	Phenotypic	Genotypic	UGA leakiness	
Amino acid 2	FC38 ⁺	X655am	N24UGA	%(FC38 ⁺ /X655am)
Leucine 1	0.006	0.28	0.29	2
Leucine 2	0.034	0.65	—	5
Leucine 3	0.043	0.60	—	7
Glycine	1.11	0.85	—	131
Cysteine	0.91	1.03	—	88
Serine	0.90	0.81	—	111
Arginine	0.11	0.22	—	50
Threonine	0.50	0.45	0.66	111
Valine	0.36	0.36	—	100
Histidine	0.44	1.03	1.44	43

b, Reduction of N24UGA leakiness by Trp-limitation

Mutant	Phage yield		
	Normal infection	Trp-limited	Trp- and Leu-limited
1. N24UGA	4.09	0 0.30	0.089
2. N24UGA	4.31 (30 °C)	0 0.39 (40 °C)	—
3. X655am (<i>supE44</i> suppressed)	2.35 (30 °C)	2 2.86 (40 °C)	—

a, The host is CP79 *relA* (λ), *supE44* at 30 °C or the restrictive temperature for the aminoacyl-tRNA synthetase mutants. The limitation for tryptophan was with 20 $\mu\text{g ml}^{-1}$ indoleacrylic acid + 20 $\mu\text{g ml}^{-1}$ 6-methyl-D,L-tryptophan, and for the second amino acid as follows: leucine 1 = auxotroph; leucine 2 = 0.50 mg ml^{-1} azaleucine; leucine 3 = *leuS*^{ts} mutant at 42 °C; glycine = *glyS*^{ts} mutant at 38 °C; cysteine = auxotroph; serine = 250 $\mu\text{g ml}^{-1}$ serine hydroxamate; arginine = auxotroph; threonine = auxotroph; valine = *valS*^{ts} at 42 °C; histidine = auxotroph. Phage yield = (phage yield at 120 min/no. of infected cells). Genotypic suppression of X655am is by the amber suppressor tRNA, *supE44*. b, The Trp-limitation for row 1 was with 20 $\mu\text{g ml}^{-1}$ indoleacrylic acid + 20 $\mu\text{g ml}^{-1}$ 6-methyl-D,L-tryptophan and for rows 2 and 3, with a *trpS*^{ts} mutant at 38 °C; the Leu-limitation was with an auxotroph.

increase the chance for a Gly-tRNA^{Gly} to read the GGC codon overlapping this position in the (−) reading frame. Were this the explanation of shiftiness at position 94–96, then limitation for Gly-tRNA should reverse the Trp-limited suppression of FC38⁺. As it does not (Table 3a), it appears that correct base-pairing in an incorrect reading frame is not the basis of phenotypic frameshifting. Rather, our evidence that a Leu-tRNA is the culprit suggests that incorrect reading in the normal reading frame is the primary event, leading to a subsequent anomaly in translocation.

A further question arises as to whether the Leu-tRNA is encoded in the bacterial or phage genome. A deletion of the phage T4 Leu-tRNA gene, *psu* Δ 33 (ref. 22), was crossed into FC38⁺ by standard phage techniques. The recombinant, FC38⁺*psu* Δ 33, displayed an identical level of suppression during Trp-limitation to FC38⁺. The Leu-tRNA is therefore encoded in the bacterial genome.

Conclusions

Tryptophan-limited suppression of (+) frameshift alleles downstream of positions 94–96 apparently reflects two consecutive

events. The first is misreading of the UGG position by a noncognate Leu-tRNA, presumably the one which normally reads UUG, favoured by the limited availability of cognate Trp-tRNA. The second is a shift into the (−) reading frame, most simply viewed as a 4-base translocation. We note that Leu-tRNA_(UGG) has already been shown to be error-prone in the third position, reading UUU with exceptionally high frequency^{23,24}. The association of incorrect translocation with incorrect tRNA recognition leads to the general conclusion that correct, 3-base translocation is not an inherent property of the ribosome, but is rather dependent on the configuration of the mRNA:tRNA match.

Misreading of UGG by Leu-tRNA_(UGG) would involve a purine:purine mispair in the second position. Precedents for such a configuration exist in the form of certain unconventional termination suppressors. The *sup46* suppressor of yeast affects the structure of ribosome protein S11 so as to permit, among other events, serine insertion at UAA positions, presumably by normal Ser-tRNA_(UCA) (refs 24–27), a purine:purine mispair in the second position.

Another case, and a particularly relevant one, is the *supK* UGA suppressor of *Salmonella*²⁸. This mutation restricts the methylation of serine- and alanine-tRNA, probably at the wobble position^{29,30}; it is presumably the undermodified Ser-tRNA_(UCA) which misreads UGA by a second position purine:purine mismatch. A striking property of *supK* is that it also suppresses certain frameshift mutant alleles³¹. Our analysis suggests that *supK* acts as a frameshift suppressor by virtue of this very mismatching propensity. Both of the frameshift alleles suppressed by *supK*, *hisD3018* and *trpA91*, generate an out-of-frame UGA codon immediately downstream of the frameshift mutation (ref. 31 and J. F. Atkins and S. Thompson, personal communication). We suggest that misreading of these UGA codons by the under-modified Ser-tRNA_(UCA) often elicits a frameshift, as in the present case, thereby leading to restoration of the correct reading frame.

The biochemical mechanism which couples codon:anticodon matching with maintenance of the reading frame remains to be elucidated. Our results are compatible with either of two distinct mechanisms. One possibility is that a mismatched tRNA in the ribosome P site, following normal translocation, distorts the geometry of the adjacent A site so that the next incoming tRNA frequently reads an out-of-frame triplet in the message. Alternatively, the initial mismatch in the A site may throw the translocation process itself out of kilter, as Kurland has suggested elsewhere on theoretical grounds^{9,32}. A general discussion of error-coupling can be found in ref. 9.

A further question arises from the fact that some positions—like the UGG codon we have considered here—are much more prone to phenotypic frameshifting than are others. There are apparently context rules governing shiftiness, analogous perhaps to context effects on nonsense suppression^{33–37}. An analysis of these context rules, based on the experimental approach followed here, will be reported elsewhere (R.W. and J.G., manuscript in preparation).

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***Drosophila* muscle myosin heavy chain encoded by a single gene in a cluster of muscle mutations**

Sanford I. Bernstein[‡], Kaname Mogami^{*}, J. James Donady[†] & Charles P. Emerson Jr^{*}

^{*}Department of Biology, Gilmer Hall, University of Virginia, Charlottesville, Virginia 22901, USA

[†]Department of Biology, Wesleyan University, Middletown, Connecticut 06457, USA

Drosophila muscle myosin heavy chain is encoded by a single-copy gene which is transcribed during both larval and adult development. This myosin gene maps to a chromosomal locus distant from any of the actin genes, but is within a cluster of flight muscle mutations.

CONTRACTILE protein genes are expressed coordinately during myogenesis. When myoblasts fuse to form skeletal muscle fibres, contractile protein mRNAs accumulate^{1–4} and their protein products—actin, myosin heavy chain, myosin light chain, tropomyosin and troponin—are synthesized and assembled into functional myofibrils^{4–6}. Often, different isoforms of each of these contractile proteins accumulate in functionally different types of muscle^{7–10}. Thus, differential expression of members of contractile protein multigene families may be important in the development of different muscle types.

Recent genetic and molecular studies of *Drosophila* indicate that contractile protein genes expressed in flight muscle are clustered in the genome. Mogami and Hotta^{11,12} isolated a collection of dominant flightless mutations which interfere with the assembly of myofibrils in the indirect flight muscle. Three of these mutations map within one centimorgan of each other on the left arm of chromosome 2 and seven map to a small region on the right arm of chromosome 3. Distinct sets of myofibrillar proteins are missing or reduced in flight muscle of each of these mutants, suggesting that each represents a lesion in a different muscle gene.

Molecular studies of the *Drosophila* actin^{13,14} and tropomyosin¹⁵ genes support the genetic data that some myofibrillar protein genes are clustered in the *Drosophila* genome. Actin genes comprise a family of six homologous sequences which are dispersed at different chromosomal sites. One of these actin genes and two tropomyosin genes map to 88F on chromosome 3. This site is near or within the cluster of dominant flight muscle mutations on this chromosome. Recent data suggest that the actin gene at 88F is specifically expressed during the differentiation of adult thoracic muscle (E. Fyrberg, personal

communication). The observation that several muscle genes are clustered in the *Drosophila* genome suggests that chromosomal location may have a role in coordinated muscle gene regulation.

We report here on the isolation and chromosomal mapping of a *Drosophila* muscle myosin heavy-chain gene to investigate further the importance of contractile protein multigene families and gene clustering in coordinate muscle gene regulation. Our results show that the myosin heavy-chain protein present in larval, leg and thoracic muscle is encoded by a single gene. Although this myosin gene is not linked to any of the six actin genes, it is located at the site of a cluster of muscle mutations on the second chromosome.

Isolation and structural analysis of a *Drosophila* myosin heavy chain gene

Myosin genomic DNA segments were isolated by plaque hybridization of a *Drosophila* recombinant phage library with a *Caenorhabditis elegans* myosin heavy-chain gene probe^{16,17}. This probe included most of the protein coding sequences of the body wall myosin heavy chain of *C. elegans*. Recombinant phage plaques equivalent to eight *Drosophila* genomes (8×10^4 phage) were screened to ensure that all sequences homologous to the myosin probe were recovered. Figure 1 describes our screening method.

The *Drosophila* library screen yielded 75 recombinant phage which hybridized to the *C. elegans* myosin probe. *EcoRI* restriction enzyme digests of DNA from 12 of these were compared. We found that five of these recombinants had at least one *EcoRI* fragment of unique size. Detailed restriction enzyme and hybridization analyses indicate that the DNA inserts of these five unique myosin recombinants can be aligned as overlapping segments of a single myosin gene locus.

The RNA coding region within the *Drosophila* myosin gene locus was identified by hybridization of *Drosophila* RNA to Southern blots containing the set of five myosin gene recom-

[‡] Present address: Molecular Biology Institute and Department of Biology, San Diego State University, San Diego, California 92182, USA.

binants. Comparison between the hybridization patterns of RNA purified from late stage embryos (the stage of larval muscle synthesis) and late stage pupae (the stage of adult muscle synthesis) showed (see Fig. 1) that poly(A)⁺ RNA probes from both these developmental stages hybridize to a contiguous 22-kilobase (kb) region within the aligned myosin gene segments. The *C. elegans* myosin gene probe also hybridizes to this same RNA coding region, except for a small terminal fragment (Fig. 1).

The transcriptional orientation of the myosin gene was established by hybridization of a quail myosin cDNA clone¹⁸ to restricted myosin gene segments. The quail cDNA clone, which contains the 3' region of the myosin transcript, hybridizes only to a 6.1-kb, *EcoRI* fragment at one end of the transcribed region of the *Drosophila* gene (see arrow, Fig. 1). Nucleotide sequence analysis shows that the *Drosophila* protein encoded in this 3' gene region is homologous to the carboxy termini of both rabbit and *C. elegans* myosin (Fig. 1). We have analysed the sequence of an extensive portion of this region (S.I.B., J.J.D. and C.P.E., in preparation) which decodes into 163 contiguous amino acids followed by a termination codon at a position close to the carboxy termini of the rabbit and *C. elegans* myosin proteins. This portion of the *Drosophila* protein shares 62% amino acid sequence homology with the rabbit myosin protein and 64% homology with the decoded *C. elegans* myosin gene. These results establish that the cloned *Drosophila* gene encodes authentic myosin heavy chain.

Fig. 1 *a*, Restriction map, transcribed region, and *b*, partial sequence of the cloned *Drosophila* myosin heavy-chain gene.

Methods: *Drosophila* myosin heavy-chain gene segments were detected by plaque hybridization³¹ of a Charon 4, λ phage library of Canton S genomic DNA³² with a *C. elegans* myosin heavy-chain gene probe. This probe, which included most of the myosin protein coding region, consisted of 5.5-kb and 2.7-kb *Bam*HI restriction fragments recovered from a *C. elegans* genomic DNA clone provided by J. Karn^{16,17}. Nick-translated probes³³ were hybridized to a total of 8×10^4 *Drosophila* library plaques at 42 °C in conditions of low stringency (30% formamide, $5 \times \text{SSC}$, $1 \times \text{Dendhardt's}$, $100 \mu\text{g ml}^{-1}$ denatured salmon sperm DNA¹⁹). Hybridization solutions also contained 10% dextran sulphate to increase the efficiency of hybridization³⁴. Filter washes were at 55 °C in conditions of low stringency (two 30-min washes each of $3 \times \text{SSC}$, 0.1% SDS and $1 \times \text{SSC}$, 0.1% SDS¹⁹) to facilitate recovery of *Drosophila* myosin genes using the heterologous *C. elegans* probe. Seventy-five hybridizing plaques were detected and 12 were chosen randomly for analysis by *Eco*RI restriction digestion and agarose gel electrophoresis. Of these 12 recombinants, 5 had at least one unique-sized *Eco*RI restriction fragment and the other 7 were duplicates of one of this set. *Eco*RI restriction site maps of these 5 recombinants were constructed by standard double-digestion restriction procedures and are shown in *a*. The *Eco*RI restriction sites of these 5 recombinants can be aligned to form an overlapping set. The overlap between these segments was confirmed by two methods. *Eco*RI fragments from $\lambda 9$, $\lambda 10$ and $\lambda 36$ MHC clones were subcloned in pBR322, nick translated and hybridized to Southern blots³⁵ containing restriction digests of putative, overlapping clones. The expected regions of homology between overlapping clones were demonstrated. Also, the putative regions of overlap between $\lambda 36$ and $\lambda 9$ and between $\lambda 7$, $\lambda 9$ and $\lambda 10$ were shown to share identical restriction sites (see figure) |, *Eco*RI; □, *Bgl*III; ○, *Sal*I; ▽, *Pst*I; □, *Ava*I; ●, *Xho*I. The DNA segments of the five myosin phage recombinants can be aligned to form the continuous DNA locus shown in the composite *Eco*RI map. The open bar on this map represents the region of hybridization of restriction fragments to the *C. elegans* myosin gene probe, and the cross-hatched bar is the region of hybridization to nicked 5' kinase-labelled poly(A)⁺ RNA from late embryos (19 h post-fertilization) and late pupae (after the appearance of eye pigments)¹³. These RNA probes were prepared by treating 1.5 μg of poly(A)⁺ RNA with tobacco acid pyrophosphatase (BRL) and bacterial alkaline phosphatase (New England Biolabs) to remove 5'-terminal caps³⁶; the RNA was then randomly cleaved by mild alkaline treatment for 10 min at 90 °C in 50 mM Tris-HCl, pH 9.5, and 5' termini of the resultant fragments were labelled with polynucleotide kinase (BRL) and [γ -³²P]ATP (Amersham)³⁷. The arrow indicates a *Hind*III restriction site which splits the 6.1-kb *Eco*RI fragment into two fragments which hybridize to a quail myosin heavy-chain cDNA clone that includes the 3'-carboxy-terminal myosin coding sequences¹⁸. Hybridization of the quail 3' myosin cDNA and nucleotide sequence analysis from this *Hind*III site (see below) establish this region to be the 3'-terminal sequence of the *Drosophila* myosin gene. Assignment of the 5' border of this gene is based on blot hybridization analysis of kinase-labelled RNA to restricted DNA from a subcloned 5.5-kb *Eco*RI fragment of $\lambda 36$. These results place the 5' border of the transcribed myosin gene close to the *Eco*RI site separating the 5.5-kb and 2.0-kb *Eco*RI fragments of $\lambda 36$. We assume that a 4.5-kb intervening sequence does not extend from this site to the border of the cloned region. In *b*, the 3' *Hind*III site, in DNA from a subclone of $\lambda 10$ MHC, was ³²P-labelled using polynucleotide kinase and sequenced by Maxam and Gilbert techniques³⁷. The complementary strand sequence was derived independently by labelling and sequencing from a downstream *Sal*I site. The figure shows the nucleotide sequence of this gene upstream from the labelled *Hind*III site (underlined). The decoded *Drosophila* amino acid sequence is compared with rabbit³⁸ and *C. elegans*¹⁷ myosin heavy-chain protein sequences. Species-specific amino acid differences in this region are indicated.

There is one copy of the cloned myosin heavy-chain gene in the *Drosophila* genome

We examined further the possibility that *Drosophila* possesses a single myosin heavy-chain gene by probing blots of genomic DNA with radiolabelled myosin gene segments. To increase the probability of detecting distantly related sequences, we used low stringency hybridization and washing conditions¹⁹ which were at the T_m of a hybrid molecule with 25% mismatch, assuming a 50% G + C content²⁰. DNA segments covering the entire *Drosophila* myosin gene, including the conserved active thiol coding sequence and the 5' and 3' gene-flanking regions, were used as hybridization probes (Fig. 2a-c). We also probed with a small 3' gene fragment which lacks intervening sequences and encodes a portion of the carboxy-terminal region of the myosin protein (Fig. 2d). Finally, we utilized the *C. elegans* myosin gene as a heterologous probe which might hybridize to more distantly related myosin genes (Fig. 2e). For all probes used, we detected only genomic DNA fragments predicted from our restriction map analysis of the myosin gene recombinants, showing that no other related myosin genes exist in the *Drosophila* genome.

The chromosomal site of the *Drosophila* myosin heavy-chain gene was determined by *in situ* hybridization of myosin gene DNA segments to polytene chromosome squashes. All regions of the cloned myosin gene locus hybridized to an identical, single site at 36B on the left arm of the second chromosome

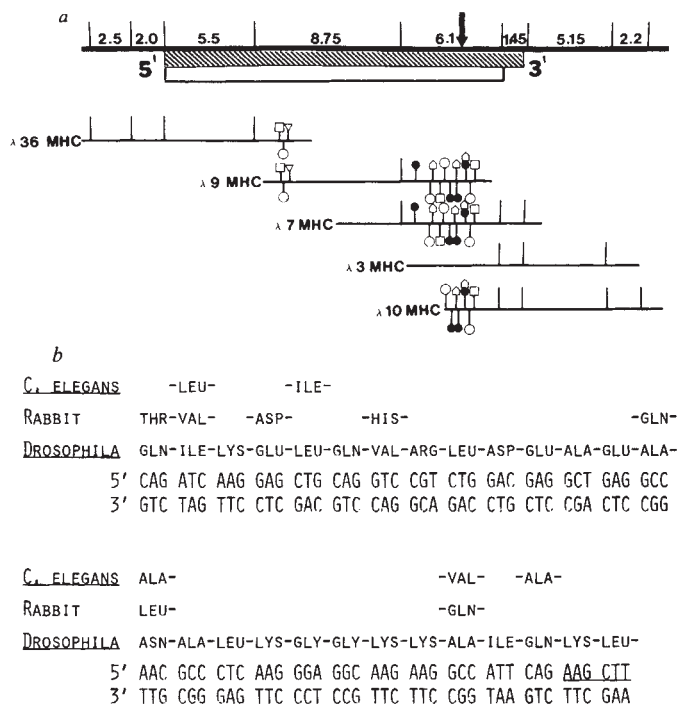
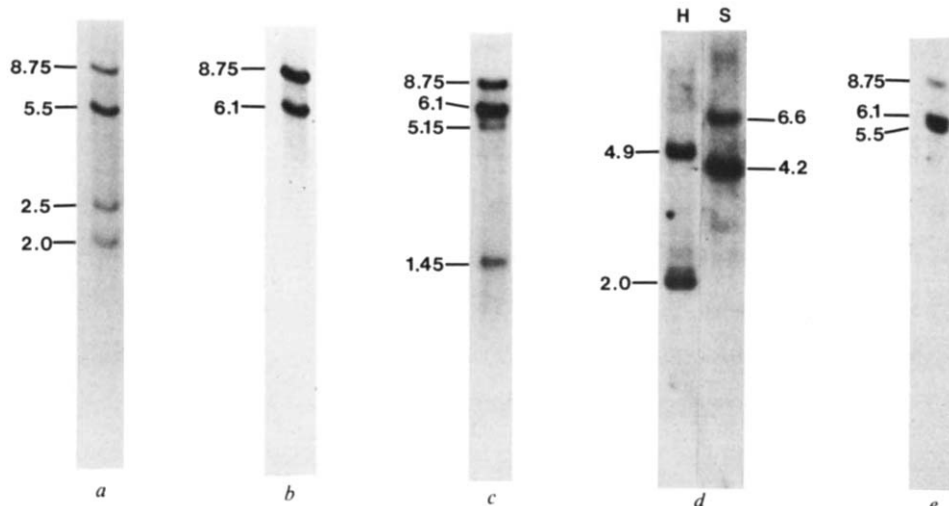


Fig. 2 Blot hybridization of *Drosophila* genomic DNA with recombinant myosin heavy-chain gene probes. *a*, *Eco*RI digest of genomic DNA probed with λ 36 MHC DNA reveals only the four expected *Eco*RI fragments of the λ 36 DNA. *b*, *Eco*RI digest of genomic DNA probed with λ 9 MHC reveals only the two expected *Eco*RI fragments of λ 9 DNA. *c*, *Eco*RI digest of genomic DNA probed with λ 7 MHC reveals only the four expected *Eco*RI fragments of λ 7 DNA. *d*, *Hind*III (H) and *Sph*I (S) digests of genomic DNA probed with a 0.45-kb *Bgl*I myosin gene fragment composed of coding region DNA. *e*, *Eco*RI digest of genomic DNA probed with the *C. elegans* myosin heavy-chain gene reveals only the three expected *Eco*RI fragments (see Fig. 1).



DNA was extracted from Canton S strain flies³⁹, digested with a restriction enzyme, and fractionated by agarose gel electrophoresis (3 μ g DNA per lane). DNA was blotted onto nitrocellulose³⁵ following treatment with HCl to enhance transfer of high molecular weight fragments³⁴. DNA blots were hybridized with various nick-translated probes in low stringency conditions (see Fig. 1) to detect putative fragments with weak homology to these probes. Sizes of hybridizing genomic DNA fragments, indicated in kilobases, were determined by comparison with those of myosin recombinant probes run in parallel on these gels (see Fig. 1). Note in *b* that the 8.75-kb fragment encodes the highly conserved active thiol portion of myosin as determined by its hybridization to this region of the *C. elegans* gene. A 2.3-kb portion of this fragment which encodes the entire active thiol region only hybridized to expected genomic restriction fragments (not shown). The *Bgl*I fragment in *c* encodes a carboxy-terminal coiled-coil portion of the myosin heavy-chain protein which shows sequence conservation among members of myosin multigene families^{26,29}. Analysis of the nucleotide sequence of the *Bgl*I fragment indicates that it encodes an uninterrupted stretch of the myosin heavy-chain protein which terminates 25 codons upstream from the putative stop codon (400 bases sequenced to date; S.I.B., J.J.D. and C.P.E., in preparation). Restriction digests of cloned DNA indicate that the enzymes *Hind*III and *Sph*I each cut once within the *Bgl*I probe, yielding the 4.9- and 2.0-kb *Hind*III fragments and 6.6- and 4.2-kb *Sph*I fragments. These are the only fragments detected on genomic blots (shown above). For all probes, similar results were obtained using several other restriction enzymes. Some weakly hybridizing bands were occasionally detected on extremely prolonged film exposure (not shown), and may represent partial digestion products, bands due to contaminating restriction enzyme activities, or sequences very distantly related to the cloned myosin heavy-chain gene.

(Fig. 3). Even after prolonged exposures (10 times the normal period), no other consistently labelled sites of hybridization were observed, further showing that the cloned myosin gene is unique in the genome.

The myosin heavy-chain gene at 36B on the second chromosome is close to or within a cluster of dominant flight muscle mutations¹¹. Mogami and Wright (in preparation) have used Y:2 translocation stocks²¹ to construct overlapping deficiencies between 34B and 40A to examine if haploidy for this region affects muscle function. They found that flies heterozygous for deficiencies between 36A1-2 and 36C1-2, as well as larger deficiencies that include this region, are flightless and have disrupted myofibrils in their indirect flight muscles.

To determine directly if the myosin gene is located within the 36AC chromosomal region, we hybridized a myosin gene fragment to blots of DNA from flies carrying one, two and three doses of 36A1-2; 36C1-2. The intensity of myosin heavy-chain gene hybridization relative to that of a gene located outside the aneuploid region varies in direct proportion to the dosage of 36AC (Fig. 4a). Therefore, the myosin gene is located in the chromosomal region which is haplo-insufficient for indirect flight muscle function.

We next examined the myosin heavy-chain protein content of various body parts of 36AC aneuploid flies. Proteins from thoraces, legs and larvae were electrophoresed on SDS-polyacrylamide gels (Fig. 4b). For all tissues examined, the myosin heavy-chain content of haploids was reduced (43–75%) relative to that of diploids. Triploids possessed 15–24% more myosin heavy chain than diploids. Thus, the content of myosin heavy chain is dependent on the dosage of the 36AC region, indicating that the myosin heavy-chain gene located at 36B is expressed in thoracic, leg and larval muscles.

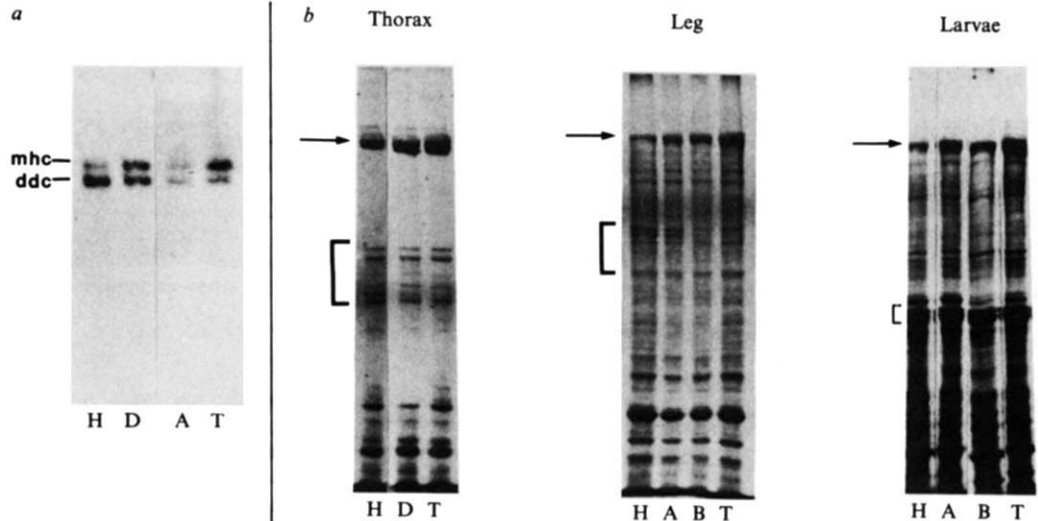
Discussion

Our study of the *Drosophila* myosin heavy-chain gene has revealed two interesting and unexpected results. We found that



Fig. 3 *In situ* hybridization of the myosin heavy-chain gene clones to salivary gland chromosomes. Tritiated cRNA was transcribed from each of the five myosin heavy-chain clones shown in Fig. 1⁴⁰, and these probes (1×10^6 c.p.m.) were hybridized to squashes of salivary gland chromosomes⁴¹. Hybridization procedures were those described by Stuart and Porter⁴² except that prehybridization RNase treatment included 20 μ g ml⁻¹ RNase A (Sigma) and 2 U ml⁻¹ RNase T1 (Sigma). Post-hybridization treatment was at twice the concentration of these RNases (E. Fyrberg, personal communication). After autoradiography and chromosome staining⁴¹, silver grains were localized to specific bands by light microscopy. Slides usually were exposed for 3 days, but for as long as 30 days to detect minor hybridization sites. The figure shows the hybridization of a λ 9 MHC probe to site 36B on the second chromosome (arrow). All cloned myosin gene probes hybridized to this 36B site. The inset shows hybridization to the 36B region of a particularly well-spread chromosome arm.

Fig. 4 *a*, Blot hybridization of genomic DNA from flies haploid, diploid and triploid for the 36A1,2 to 36C1,2 second chromosome region. *b*, Polyacrylamide gel analysis of proteins from *Drosophila* carrying haploid, diploid and triploid doses of the 36A1,2; 36C1,2 region. In *a*, DNA was isolated³⁹ from various Y:2 translocation stocks of adult *Drosophila*²¹, digested with *Eco*RI and fractionated on a 0.8% agarose gel. Blots of these gels were hybridized with a nick-translated *Eco*RI-pBR322 subclone of λ 10MHC that is homologous to the 6.1-kb *Eco*RI fragment of the myosin heavy-chain gene. Blots were also hybridized with a 5.5-kb subcloned *Eco*RI fragment of the *Drosophila* dopa decarboxylase (*Ddc*) gene⁴³. The *Ddc* gene is located at 37C⁴⁴, outside the 36AC aneuploid region, and serves as an internal hybridization control to compare relative hybridization intensities on autoradiographs of these blots. The figure shows autoradiographs of a blot hybridization analysis of DNA from wild type and aneuploid fly stocks constructed by Mogami and Wright (in preparation). The aneuploid stocks are translocation heterozygotes produced by crossing Y:2 translocation stock A62 (having a breakpoint at 36C1,2) with stock B214 (having a breakpoint at 36A1,2-6,7)²¹. D, diploid Canton-S, wild-type DNA; A, parental (A62) translocation stock; (the B214 translocation stock yields an identical pattern); H, haploid for the 36AC region; T, triploid for 36AC region. In the diploid (D) and parental translocation stock (A) DNAs, the relative hybridization intensities of the 6.1-kb myosin fragment (*mhc*) and the 5.5-kb dopa decarboxylase (*ddc*) fragments are equivalent. In the haploid translocation stock (H), hybridization to the myosin gene is reduced relative to the *Ddc* gene; and in the triploid translocation stock (T), hybridization to the myosin gene is increased relative to the *Ddc* gene. These differences in relative myosin and *Ddc* gene hybridization were confirmed by scanning densitometry of autoradiographs. The ratio of hybridization of *mhc*:*ddc* for haploids = 0.5; diploids = 1.0; parentals = 1.1; triploids = 1.4. In *b*, one fly thorax, six pairs of legs or one larva (third instar) were homogenized in a microtissue grinder in 20–40 μ l of SDS sample buffer⁴⁵. The samples were boiled, debris was pelleted and 1–15 μ l of each sample was fractionated by electrophoresis on a 7.5% polyacrylamide gel containing SDS⁴⁵. Gels were stained with Coomassie brilliant blue and analysed by densitometry. The arrow indicates the location of putative myosin heavy chain. the bracket denotes the proteins used as internal standards to allow comparison of the quantity of myosin heavy chain present in the different genotypes. The ratio of myosin to the internal standards are: for thoracic proteins: H (haploid) = 0.48, D (diploid, Canton-S) = 0.84, T (triploid) = 1.04; for leg proteins: H (haploid) = 0.20, A (diploid, parental A62) = 0.76, B (diploid, parental B214) = 0.82, T (triploid) = 0.91; for larval proteins: H (haploid) = 0.28, A (diploid, parental A62) = 0.69, B (diploid, parental B214) = 0.60, T (triploid) = 0.76. The ratio of myosin heavy chain to other proteins is clearly less in all tissues examined for haploids as compared to diploids and triploids for the 36AC region.



Drosophila muscle myosin heavy chain is encoded by a single gene rather than a multigene family. In addition, this myosin gene maps to a chromosomal region which contains a cluster of flight muscle mutations and is haplo-insufficient for flight muscle function.

The finding of a single muscle myosin heavy-chain gene was unexpected, because other organisms have multigene families which encode tissue- and stage-specific isoforms of myosin heavy chain^{22–26}. Like these other organisms, *Drosophila* has several muscle types which exhibit ultrastructural and physiological differences. For example, tubular muscles and indirect flight muscles differ in regard to the regularity of their filament organization, their thick:thin filament ratio and their response to excitation²⁷. There is also evidence for myosin ATPase heterogeneity in *Drosophila*²⁸. All of these properties could be influenced by the presence of different forms of myosin heavy chain.

Our conclusion that *Drosophila* possesses one myosin heavy-chain gene is supported by several lines of evidence. Only one myosin gene was isolated from the recombinant clone library. Of 12 clones examined, all are derived from this single myosin gene, indicating that it is very unlikely ($P < 0.00025$) that other homologous genes exist in the recombinant library. *In situ* hybridization and genomic blotting experiments using DNA fragments covering the entire myosin gene revealed only one such gene in the *Drosophila* genome. DNA blots probed with the highly conserved active thiol coding region¹⁷, a conserved 3' coding sequence^{26,29}, and the *C. elegans* myosin gene yielded hybridization patterns identical to the cloned *Drosophila* myosin gene (Fig. 2). We should have detected all sequence-

related myosin genes because our blot hybridization and washing conditions were considerably less stringent than those at which other myosin heavy-chain multigene families cross-hybridize^{22,23,26}. However, distantly related non-muscle myosin genes may not have hybridized to our probes in these conditions^{22,26}. We obtained additional proof that *Drosophila* possesses a single muscle myosin heavy-chain gene by showing that RNA homologous to the cloned gene is present at both larval and pupal stages of *de novo* muscle synthesis. By analysing segmental aneuploids, we determined that the myosin encoded at the 36B locus is present in larval, leg and thoracic muscles. These results suggest that additional genes encoding muscle myosin heavy chain are not required in *Drosophila*.

Although a single *Drosophila* myosin gene is expressed in larval, leg and thorax muscles, this gene may encode multiple forms of myosin heavy-chain protein. This possibility is suggested by the presence of three species of polyadenylated myosin transcripts in late embryonic and late pupal stage RNA (ref. 30 and S.I.B., J.J.D. and C.P.E., in preparation). One RNA size class is unique to each stage and one is shared. We are now testing the hypothesis that different modes of RNA transcription or processing result in the production of transcripts encoding stage- or muscle-specific forms of the myosin protein.

Our other interesting finding is that the myosin heavy-chain gene maps to a chromosomal region which contains a cluster of flight muscle mutations and is haplo-insufficient for flight muscle function. The reduced levels of myosin heavy-chain protein present in the thoraces of flies haploid for the 36A1,2; 36C1,2 chromosomal region may result in loss of flight ability. However, larval and leg muscle, which also display reduced

levels of myosin in haploids, seem to function normally. Perhaps the functional integrity of these non-flight muscles is insensitive to a twofold variation in myosin heavy-chain protein content. Alternatively, another gene in the 36B region which is transcribed only in flight muscle may be responsible for the abnormalities in haploids.

Localization of the myosin gene to the cluster of muscle mutations at 36B, as well as mapping of an actin and two tropomyosin genes to a second cluster of mutations at 88F, support the hypothesis that these mutations are in structural protein genes¹¹. The precise location of these mutations and the extent of muscle gene clustering at 36B and 88F are unknown. To examine these questions and to investigate whether there is a link between muscle gene clustering and

coordinate gene regulation, we are isolating and characterizing the genes linked to the myosin heavy-chain gene locus.

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LETTERS TO NATURE

A concentration of quasars in the Sculptor region of the sky

Halton Arp

Mount Wilson and Las Campanas Observatories of the Carnegie Institution of Washington, Pasadena, California 91101, USA

The strip of sky 5° wide and centred at dec -40° is the largest area of the sky which has been completely searched for quasars¹. The strip is long and narrow and runs from RA ≤ 22 h to RA ≥ 4 h. Near the centre of the strip, at 0 h < RA < 1 h, there is an evident concentration of quasars. Osmer² and Smith³ have argued that this concentration is caused by the sensitivity of the plates taken at larger and smaller right ascensions being less than those taken in 0 h < RA < 1 h region. In the original report of the survey, there was no note that any regions showed systematically poorer seeing or lower sky-density for a given exposure. It seems necessary to substantiate such an *a posteriori* hypothesis with quantitative systematic differences in the plate quality. In this report the reality of this concentration has been tested by asking whether all quasars show this concentration or only certain kinds of quasars.

The objective prism Curtis Schmidt plates on which these quasars were selected were sensitive from about λ 3,400 Å of atmospheric UV transmission to λ 5,400 Å of the IIIA-J plate cutoff. The strongest emission line visible in this window is what marked the object as a quasar. We can take the strength of this line as measured in equivalent width, *W*, from the tabulation by Osmer and Smith¹. Usually, this is Lyman α 1,215.7 in the redshift range above *z* ≈ 1.9. We define a strong line quasar which has for its strongest line in the objective prism window *W* ≥ 220 and a weak line quasar one which has *W* < 220. (The 220 Å equivalent width was chosen because it roughly divides

the sample in half.) In 11 cases (listed in Table 1) Osmer and Smith had not measured the equivalent width of the principal line in the detection window. Table 1 estimates these missing values of *W* from the published spectra and/or the correlation between the equivalent width of C IV and Lyα.

In the bottom two histograms of Fig. 1 we plot the distribution of the weak line quasars and the distribution of the strong line quasars. It is seen that the concentration between 0 h < RA < 1 h is not present for the weak line quasars, but is markedly present for the strong line quasars. The concentration of strong line quasars can be seen most dramatically in the ratio of strong to weak line quasars in the third histogram from the bottom.

Table 1 Quasars with a strongest line in objective prism window which had no measured equivalent width (*W*)

Quasar	<i>z</i>	<i>W</i> from (spectrum)	<i>W</i> from (C IV)	Decision	Note
0004-408	2.09	200	140	Weak	
0018-388	2.29	330	220	Strong	
0032-413	1.54	~220	—	Omit	
0222-415	2.00	180	100	Weak	
0224-419	2.13	180	200	Weak	
0238-382	2.26	300	140	Strong	
0304-392	1.97	—	170	Weak	Ly not in spectrum
0326-403	2.10	—	180	Weak	Ly not in spectrum
0338-405	2.08	200	140	Weak	
0353-383	1.96	—	120	Weak	Ly on edge of spectrum
0447-395	1.98	—	200	Weak	Ly on edge of spectrum

2351-406 was omitted from analysis because it is anomalously faint, with a large value for *W*.

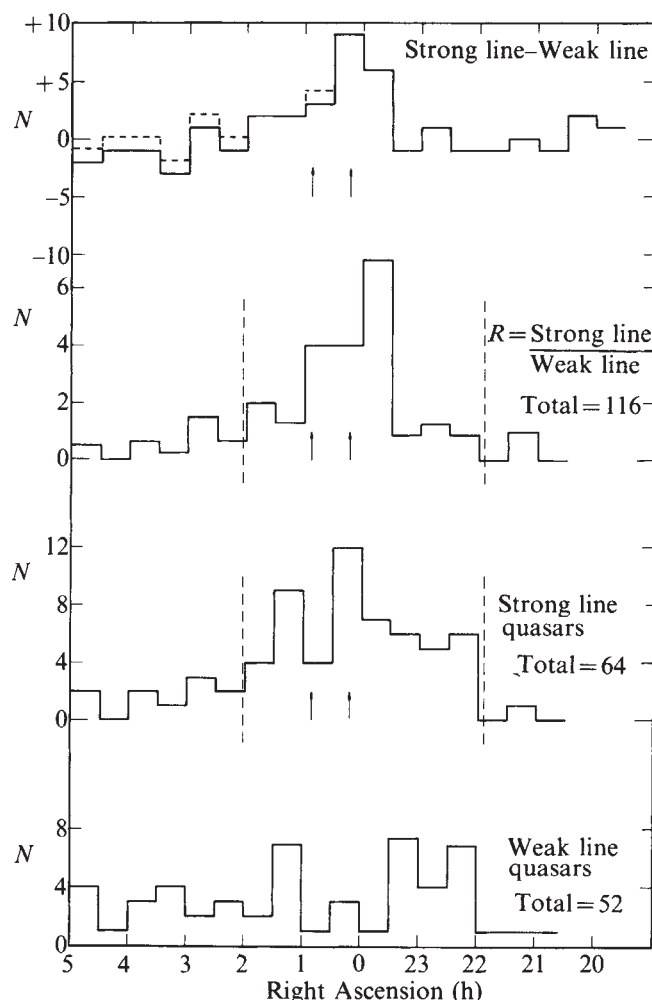


Fig. 1 Frequency distributions of quasars in the dec -40° zone. The strip is 5° wide in dec and runs from RA < 20 h to RA > 5 h. Strong line quasars are defined as having the equivalent width of the strongest line in the detection window $W \geq 220$. The rest are defined as weak line. Vertical dashed lines indicate regions greater than which and less than which there has been some question about survey completeness. In the upper histogram dashed bins indicate correction to strong line quasar numbers needed to bring agreement with net numbers at RA < 23 h 30 min. Vertical arrows indicate position of NGC55 and NGC300, two of the largest galaxies in the Sculptor group.

As for the postulated systematic effects with right ascension, the evenness of distribution for the weak line quasars indicates the search is uniformly complete for this kind of quasar throughout the strip. At any given continuum magnitude, the weak line quasars are the most difficult to detect and hence the first to be lost in the case of an hypothesized reduction in plate sensitivity. (This also argues against a galactic absorption effect, and, in any case, galactic absorption would be small at these latitudes with the increase of quasars to fainter magnitudes not steep enough to give a significant effect.) There is one scenario which could explain the concentration of strong line quasars, however. If we postulate that the plates Osmer called insensitive, were instead, for some reason, all taken in bad seeing or poor focus, all the bright quasars would have broadened spectra in which weak lines were more easily detectable. But the fainter quasars would be lost. Since the quasars here obey the Baldwin^{4,5} relation that stronger line quasars are fainter in apparent magnitude, such effects could change the ratio of strong to weak line quasars discovered on a plate. The magnitude limit does seem brighter for plates with RA > 2 h and we will attempt a correction for that in a moment. If we just exclude all questionable areas, however (outside the dashed vertical lines in Fig. 1), we still see the concentration of quasars to the

Table 2 Widths of spectra on and off Sculptor region

Range	No. of plates	Average spectral width
21 h 54 min–23 h 30 min	$3\frac{1}{2}$	2.4
23 h 30 min–01 h 30 min	$4\frac{1}{2}$	2.4
01 h 30 min–02 h 30 min	3	2.5

0 h $< RA < 1$ h region. It would seem that unless just the Sculptor region plates were taken under unusually good seeing or unrepresentatively sharp focus that the concentration in the 0 h $< RA < 1$ h region has to be accepted as real. If the widths of the spectra on the Curtis Schmidt plates in this survey were now measured, and the regions in the 0 h $> RA < 1$ h range shown to be not exceptionally narrow, this concentration of quasars could be demonstrated to be real without further question.

The widths of the spectra have, however, been measured by Smith (ref. 3, Table 1.3). As is shown in Table 2 here, the spectrum widths in the Sculptor region average almost exactly the same as in regions at immediately earlier and later right ascensions.

Another way of checking for the effect of survey inhomogeneities is to plot apparent magnitude-observed equivalent width diagram for all the Curtis Schmidt quasars. In that diagram we can, for example, exclude from consideration all quasars fainter than mag = 18.5 for $W = 180$ to mag = 20.0 for $W = 550$ (along a line of approximate signal-to-noise constancy). Working with the remaining 80 quasars which have the highest signal-to-noise spectra, and hence form the most complete part of the sample we get the results shown in Table 3.

Table 3 Highest signal-to-noise spectra

Range	No. strong/weak	Ratio
20 h 00 min–23 h 30 min	$S/W = 13/12$	~ 1
23 h 30 min–01 h 30 min	$S/W = 23/5$	~ 5
01 h 30 min–04 h 56 min	$S/W = 14\frac{1}{2}/13\frac{1}{2}$	~ 1

Perhaps the most interesting result of all is that there is no clear Baldwin relation, mag versus $\log W_0(C IV)$, off the Sculptor region for these Curtis Schmidt quasars but a very clear relation for quasars which are actually on the position of the Sculptor Group. If the quasars are at their redshift distance we should see a Baldwin relation in all directions. We do not. But if a group of quasars all at the same distance is encountered, as in the Sculptor region, we will see whatever intrinsic luminosity–line width relation they actually have regardless of the distribution of their redshifts.

In addition to these measures, however, there are three independent checks which confirm the reality of the concentration. One is presented in the top histogram of Fig. 1 which shows the numbers of strong-line minus the number of weak-line quasars. At RA < 23 h 30 min this difference is almost exactly zero. If we assume this is characteristic of the average field of this search, we find we need to add seven strong line quasars to the regions at RA ≥ 2 h, or about 1.1 quasars per bin. The dashed histogram indicates the rise in the net numbers of strong line quasars, including one of the areas in this series of plates at RA = 00 h 54 min 47 s. The concentration is still well marked. Second, there is evidence to show^{6,7} that the $z \geq 2.5$ quasars are more dense around NGC55 and NGC300. (The positions marked by the arrows in Fig. 1.) These high redshift quasars tend to be brighter in apparent magnitude and less subject to any plate limit effects. Finally, the CTIO grism plates taken by Hoag and Smith and analysed by Osmer provide supporting evidence. Nine grism plates were taken in the region RA = 21 h 12 min to 22 h 48 min and 30 quasars were found. Six grism plates were taken in the region RA = 00 h 02 min to 01 h 30 min and 33 quasars were found. The density in the

latter region is significantly higher:

$$\begin{aligned}\rho &= 11.1 \pm 2.0 \text{ quasars deg}^{-2} \text{ off Sculptor region} \\ \rho &= 18.3 \pm 2.4 \text{ quasars deg}^{-2} \text{ on Sculptor region} \\ &[\text{uncertainty calculated from } \pm \sqrt{n}]\end{aligned}$$

Plates taken at a different time with a completely different telescope and different dispersing element confirm the high density of quasars in the region in question. It should also be noted that Clowes⁸ has independently found that the quasar density is in fact high in this grism-objective prism overlap region.

The consequences of this concentration are very important indeed. The redshift for this group of strong line quasars run from about $1.9 < z < 2.9$. This is much too large a spread for quasars belonging to the same or even adjacent superclusters⁹. On the cosmological hypothesis of redshift distances, their small extension across the sky conflicts with their large extension in depth.

Interestingly the concentration is one of strong line quasars. Therefore, it is a particular kind of quasar which is grouped. These kinds of quasars are, by the Baldwin relation, the least luminous quasars. In the local hypothesis, they would become the lowest luminosity quasars if assigned to the near distance of the Sculptor group of galaxies^{6,7}. NGC55 and NGC300, two of the largest Sculptor group galaxies, are marked by vertical arrows in Fig. 1. If this grouping of quasars violates the cosmological hypothesis, it seems more attractive to consider the evidence which has been put forward for detailed association with the nearby Sculptor group galaxies^{6,7,10}.

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Comets, Planet X and the orbit of Neptune

M. E. Bailey

Astronomy Centre, University of Sussex, Brighton BN1 9QH, UK

The recent discovery that Pluto's mass is negligible compared with that of the Earth¹ has raised again the question of the cause of the important discrepancies between the observed and computed positions of the outer planets^{2,3}. The unmodelled force appears to require a mass of $\approx 1M_{\oplus}$ at a distance of order 50 AU from the Sun, but the non-discovery^{4,5} of any single body of this size puts the 'Planet X' hypothesis in severe difficulty. Recent work on cometary theory⁶ has shown, however, that in order for the Oort cloud to survive the strong perturbations induced by close encounters with giant molecular clouds^{7,8} it is necessary to postulate a much more centrally condensed comet cloud than Oort⁹ originally described. Thus a measurement of the cometary space density just beyond the planetary system could provide a crucial test of interstellar and primordial theories of comet origins. One way by which this could be done was suggested previously¹⁰. Here I have modified an earlier suggestion by Whipple^{11,12} and propose that a dense inner core for the Oort cloud could provide the unmodelled force which perturbs the planets.

Theories of Solar System origin cannot yet predict reliably the mass of a hypothetical shell of comets lying just beyond the planetary system ($r \approx 50$ – 100 AU, say), but in principle its mass could be quite large. Both Whipple¹³ and Hills¹⁴ have estimated that as much as $\approx 10^{-2} M_{\odot}$ could exist in this region, yet remain undetected by present observations, and extrapolation of standard Oort cloud theory into regions of small radii also hints at surprisingly high cometary space densities at $r \approx 10^2$ AU from the Sun. For example, if the number of comets in the cloud with energies $E = -GM_{\odot}/2a$ in the range $(E, E + dE)$ is $N(E)dE = k\epsilon^{-q}dE$, where $\epsilon = E/\bar{E}$ and $\bar{E} = -GM_{\odot}/2R_0$, $R_0 \approx 10^5$ AU being the cloud's outer radius, then it can be shown (see ref. 6) that close to the planetary system ($r \ll R_0$) the space density $n(r)$ is

$$n(r) = \frac{2^{2-q}}{\pi^2} B\left(\frac{7}{2}-q, \frac{3}{2}\right) kGM_{\odot}R_0^{-4} \left(\frac{R_0}{r}\right)^{4-q} \quad (1)$$

where $B(\mu, \nu)$ is the usual beta-function. In the standard Oort model of the cloud⁹ the index q of the energy spectrum is $\frac{5}{2}$, but observations and theoretical ideas^{6,14} suggest that in fact smaller values of q should apply. The normalizing constant k can be determined empirically from the observed near-parabolic flux of comets⁶, and assuming an influx of the order of 1 new comet per yr per AU with $a \geq 3 \times 10^4$ AU equation (1) becomes

$$n(r) \approx 10^{5-2q} 3^{4-q} 2^{2-q} \pi^{-1} (7-2q) \times B\left(\frac{7}{2}-q, \frac{3}{2}\right) r_{100}^{q-4} \text{ AU}^{-3} \quad (2)$$

where $r_{100} = r/100$ AU. In the standard model ($q = \frac{5}{2}$) $n(100 \text{ AU}) \approx 1 \text{ AU}^{-3}$, but for smaller values of q the density rises steeply. Hills¹⁴ has argued that q should be negative; and setting $q = -\frac{1}{2}$ in equation (2) gives $n(100 \text{ AU}) \approx 2 \times 10^8 \text{ AU}^{-3}$. Thus on both theoretical and observational grounds it is possible that a dense inner core to the Oort cloud could contain a substantial cometary mass. The detection or otherwise of such a core would lead to important constraints on theories of cometary and Solar System origin.

It is well-known that the gravitational attraction within the space enclosed by a shell of matter vanishes provided the iso-density surfaces are similar ellipsoids. But this condition is unlikely to apply to the comet shell, because on general grounds one should expect primordial comets to have a much flatter distribution closer to the planetary system. Thus (contrary to Whipple's statement¹³) a symmetrical shell of comets could affect the planets. Here we assume for simplicity that the iso-density surfaces are oblate spheroids, and calculate the forces F_{ω} and F_z parallel and perpendicular to the equatorial plane of the spheroids respectively.

The gravitational acceleration within an infinitesimal oblate spheroidal shell of density $\rho(a)$ bounded by spheroids with semi-major axes $(a, a+da)$ and eccentricities $(e, e+de)$ is given¹⁵ by $dF_{\omega} = -2\pi G\bar{\omega}(A(e+de) - A(e))\rho(a)$ and $dF_z = -4\pi Gz(B(e+de) - B(e))\rho(a)$, where $\bar{\omega} = (x^2 + y^2)^{1/2}$ is the equatorial radial coordinate and z is measured along the minor axis. Here

$$B(e) = 1 - A(e) = e^{-3} \sqrt{1-e^2} \left(\frac{e}{\sqrt{1-e^2}} - \sin^{-1} e \right) \quad (3)$$

The acceleration at a point inside a finite shell, bounded by spheroids of semi-major axes a_1 and a_2 with eccentricities $e(a_1)$ and $e(a_2)$ ($a_1 < a_2$) is therefore

$$\begin{aligned}F_{\omega} &= -2\pi G\bar{\omega} \left\{ [B\rho]_{a_2}^{a_1} + \int_{a_1}^{a_2} B \frac{d\rho}{da} da \right\} \\ F_z &= +4\pi Gz \left\{ [B\rho]_{a_2}^{a_1} + \int_{a_1}^{a_2} B \frac{d\rho}{da} da \right\}\end{aligned} \quad (4)$$

The mass of the cometary shell is

$$M_c = \int_{a_1}^{a_2} 4\pi \rho(a) a^2 \sqrt{1-e^2} da \quad (5)$$

so the forces can be written in the convenient parametric form $(F_\varpi, F_z) = (\alpha\varpi, \beta z)$ where

$$\alpha = -\frac{GM_c}{a_1^3} \frac{1}{2} \frac{I}{J} = -\frac{1}{2}\beta \quad (6)$$

and the dimensionless quantities I and J are just integrals over the density and eccentricity distributions of the material in the shell. From (4) and (5), with $x' = a/a_1$ and $\rho^* = \rho/\rho(a_1)$, we have

$$I = [B\rho^*]_{x_2}^{x_1} + \int_{x_1}^{x_2} B \frac{d\rho^*}{dx'} dx' \quad (7)$$

$$J = \int_{x_1}^{x_2} \rho^*(x') x'^2 \sqrt{1-e^2} dx' \quad (8)$$

Note that $I = 0$ for distributions with constant eccentricity.

The effect of such a comet shell on a planet's orbit can be readily obtained by solving the usual planetary equations (for example, ref. 16, section 14.04). Following Whipple¹¹ it is convenient to define u to be the mean longitude of the planet measured from its ascending node at the ecliptic and Ω to be the angle from the ecliptic node to the ascending node with respect to the $z = 0$ plane defined by the cometary mass distribution. If the planet is now assumed to have a circular orbit of radius r inclined at an angle i to the $z = 0$ plane, the height z of the planet above the plane is $z = r \sin i \sin(u - \Omega)$. If, further, i is assumed to be small (so that terms of order z^2/r^2 can be neglected), the acceleration W perpendicular to the orbital plane can be written

$$W \approx -\frac{z}{r} F_\varpi + F_z = +\frac{3}{2} \frac{GM_c}{a_1^3} \frac{I}{J} z \quad (9)$$

This may be compared with the corresponding expression due to a comet ring of mass M_c and radius a_c , given (see ref. 11) by

$$W = -\frac{GM_c}{a_c^3} \frac{b_{3/2}(p)}{2p} z \quad (10)$$

where $p = r/a_c$ and the Laplace coefficient $b_{3/2}(p)$ is given in terms of the complete elliptic integrals $K(p)$ and $E(p)$ of the first and second kinds respectively by

$$b_{3/2}(p) = \frac{4}{\pi p} \frac{1}{(1-p^2)} \left[\frac{(1+p^2)}{(1-p^2)} E(p) - K(p) \right] \quad (11)$$

Forces of the general form of equations (9) and (10) can be rewritten in the form of Whipple's equation (6) (note the typographical error in ref. 11: a should read a_p):

$$W = -2n^2 r K \sin(u - \Omega) \quad (12)$$

where $n^2 = G(M_\odot + M_p)/r^3$, M_p is the planet's mass and K is a constant which can be estimated directly from an analysis of the residuals in the planet's geocentric orbital latitude (for example, ref. 11). Using residuals determined by Wylie¹⁷ for Neptune, Whipple concluded $K = +0.28 \pm 0.12$ arc s, where the error is approximate. However, an effect due to non-zero K is extremely difficult to separate from effects due to improvements in Neptune's orbital elements. Thus, because Whipple's solution was based on a now out-dated Solar System model, it does not rule out the possibility that a new analysis, based on residuals from an orbit using the best recently determined planetary masses and orbital elements, would result in a negative value for K . In this way an estimate of the quantity

$$K_{\text{shell}} = -\frac{3}{4} \frac{M_c}{(M_\odot + M_p)} \sin(i) \left(\frac{r}{a_1} \right)^3 \frac{I}{J} \quad (13)$$

could provide a constraint on the mass M_c of a dense flattened inner core to the Oort cloud. We note that M_c cannot be determined by a single measurement of K_{shell} , but further constraints on the unknowns M_c , $\sin i$, a_1^{-3} and I/J can in principle be provided by analyses which also incorporate data from the other outer planets. It is possible too that the orbits of certain periodic comets such as P/Halley might prove useful¹⁸, provided that corrections due to non-gravitational forces can be made reliably.

In the general case an analysis for an extra force of the form $(F_\varpi, F_z) = (\alpha\varpi, \beta z)$ would be most helpful. If this were done not only could theories of cometary origin be constrained in a completely new fashion, having important implications for the still-continuing 'Solar System versus interstellar' debate, but also the results would be of interest for theories of the origin of the Solar System itself. In this way the anomalous residuals of Neptune's orbit might provide a key to an understanding of the origin of the Sun and planets.

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Nd and Sr isotopes in kimberlites and lamproites from Western Australia: an enriched mantle origin

M. T. McCulloch*, A. L. Jaques†, D. R. Nelson* & J. D. Lewis‡

* Research School of Earth Sciences, The Australian National University, Canberra, ACT 2600, Australia

† Bureau of Mineral Resources, PO Box 378, Canberra City ACT 2601, Australia

‡ Geological Survey of Western Australia, 66 Adelaide Terrace, Perth 6000, Western Australia

Diamond-bearing kimberlites and lamproites from the Fitzroy area of the West Kimberley region of Western Australia have isotopic compositions ($\epsilon_{\text{Nd}} = -7.4$ to -15.4 , $^{87}\text{Sr}/^{86}\text{Sr}$ (I) = 0.7104 to 0.7187) indicating derivation from ancient ($> 1,000$ Myr) highly enriched (high Rb/Sr, Nd/Sm) mantle sources. The Nd and Sr isotopic ratios of the kimberlites overlap those of the lamproites, consistent with the petrological and geochemical gradation between the two groups, and implies a genetic relationship. Mixtures of highly enriched mantle like that reported here with depleted MORB-type mantle can, in principle, account for both the enriched and depleted portions of mantle array. Here we present new Nd and Sr isotopic data for a suite of kimberlites and associated lamproites from the Fitzroy area which indicate that the magmas were derived from extremely enriched (high Rb/Sr, Nd/Sm) mantle and cast doubt on the validity of a primitive chondritic Sm/Nd source for kimberlites. We also present a model which accounts for the apparent clustering of many kimberlites near $\epsilon_{\text{Nd}} \sim 0$, and for the covariation of Nd and Sr isotopes in oceanic basalts (that is, the mantle array).

Previous Nd isotopic studies^{1,2} of kimberlites have shown that they generally have initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratios corresponding to $\epsilon_{\text{Nd}} \sim 0$, implying derivation from a relatively undifferenti-

Table 1 Nd and Sr isotopic data for kimberlites and lamproites from the West Kimberley region, Western Australia

Locality	Samples	Rb	Sr (p.p.m.)	Sm	Nd	$^{87}\text{Rb}/^{86}\text{Sr}$	$^{87}\text{Sr}/^{86}\text{Sr}$	$^{147}\text{Sm}/^{144}\text{Nd}$	$^{143}\text{Nd}/^{144}\text{Nd}$	$^{87}\text{Sr}/^{86}\text{Sr(I)}^*$	ϵ_{Nd}^*	$T_{\text{DM}}^{\dagger}$ (Myr)
Mt North	WAK-2-L†	350	1,481	25.3	213.1	0.682	0.71404±5	0.0719	0.51108±4	0.71385	-14.4	1,260
Oscar Plug	WAK-6-L	3,400	997	15.9	131.8	9.855	0.72008±5	0.0729	0.51104±3	0.71728	-15.2	1,310
Mr Percy	WAK-10-L	668	1,845	37.4	336.5	1.046	0.71646±4	0.0672	0.51112±2	0.71616	-13.7	1,190
Fishery Hill	WAK-14-L	258	1,546	15.2	129.4	0.482	0.71879±5	0.0710	0.51115±2	0.71865	-13.1	1,190
Walgidee Hill	WAK-25-L	477	1,904	13.1	97.0	0.723	0.71254±5	0.0816	0.51130±2	0.71233	-10.2	1,110
	WAK-30-L	490	1,323	11.1	88.5	1.070	0.71262±4	0.0759	0.51140±2	0.71232	-8.2	970
Ellendale	WAK-15-K	569	1,001	16.1	154.8	1.642	0.71114±4	0.0629	0.51117±2	0.71067	-12.7	1,110
	WAK-16-K	347	991	10.9	89.4	1.011	0.71066±5	0.0740	0.51142±3	0.71037	-7.8	940
	WAK-17-L	1,340	1,149	17.2	147.9	3.370	0.71773±4	0.0703	0.51103±2	0.71677	-15.4	1,300
	WAK-20-K	444	1,531	22.3	207.5	0.838	0.71250±9	0.0650	0.51111±3	0.71226	-13.8	1,180
	WAK-21-K	537	1,512	24.0	222.5	1.026	0.71271±4	0.0653	0.51119±2	0.71242	-12.3	1,110
	WAK-22-K	388	1,057	10.7	87.4	1.060	0.71085±6	0.0739	0.51144±2	0.71055	-7.4	920
	WAK-27-L	313	1,526	19.0	160.4	0.593	0.71499±5	0.0717	0.51111±3	0.71482	-13.9	1,230

* ϵ_{Nd} and $^{87}\text{Sr}/^{86}\text{Sr(I)}$ calculated at 20 Myr.† $T_{\text{DM}}^{\dagger} = \frac{1}{\lambda_{\text{Sm}}} \ln \left[1 + \frac{(^{143}\text{Nd}/^{144}\text{Nd})_{\text{meas}} - (^{143}\text{Nd}/^{144}\text{Nd})_{\text{DM}}^0}{(^{147}\text{Sm}/^{144}\text{Nd})_{\text{meas}} - (^{147}\text{Sm}/^{144}\text{Nd})_{\text{DM}}^0} \right]$ where $(^{143}\text{Nd}/^{144}\text{Nd})_{\text{DM}}^0 = 0.51235$ and $(^{147}\text{Sm}/^{144}\text{Nd})_{\text{DM}}^0 = 0.225$.

‡ L, Lamproite; K, kimberlite.

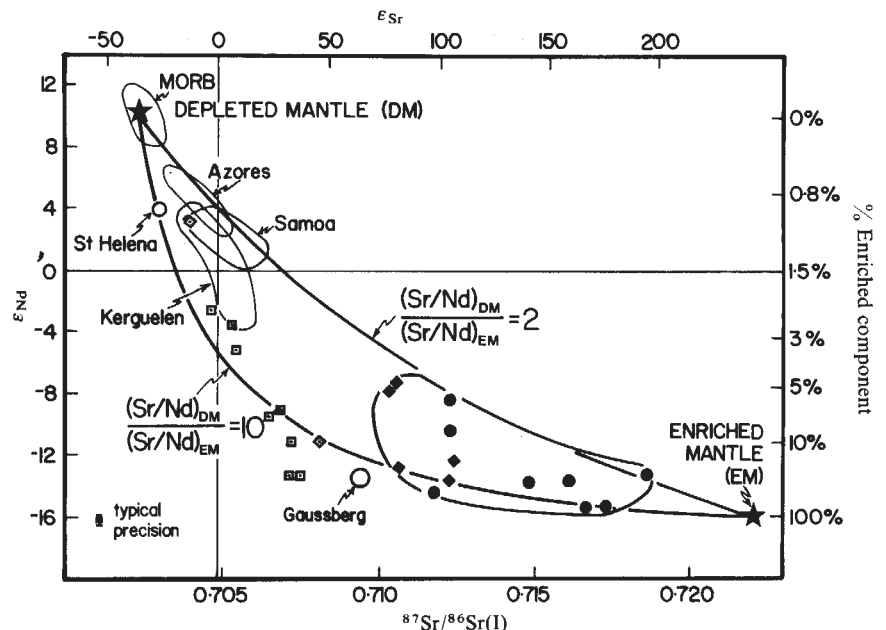
Petrological and locality data are given in ref. 10; analytical procedures are described in ref. 39.

ated mantle source with chondritic Sm/Nd. This interpretation is difficult to reconcile with the fact that many kimberlites have relatively radiogenic $^{87}\text{Sr}/^{86}\text{Sr}$ (0.704–0.709) (refs 3, 4), and evolved oceanic-island-type Pb isotopic compositions⁵. It is also inconsistent with rare-earth element (REE) abundances in kimberlites which suggest that they are derived from a light (L) REE-enriched mantle source⁶. Furthermore, Nixon *et al.*⁷ have proposed that kimberlites are hybrid melts, resulting from admixing of melts derived from deeper 'fertile' (asthenospheric) mantle with melt enriched in 'incompatible' elements at the base of the lithosphere.

The recently discovered diamond-bearing kimberlitic rocks of the West Kimberley region of Western Australia occur intimately associated with the leucite lamproites of the Fitzroy area described by Prider^{8,9}. Together they form a broad belt comprising more than 100 separate diatremes, plugs, sills and dykes, and have been dated as early Miocene (20 Myr)^{10–12}. The West Kimberley rocks show a petrological and geochemical gradation from phlogopite kimberlite (40–45% SiO_2 , >20% MgO) through olivine-rich, leucite-poor lamproite to leucite lamproite (>50% SiO_2 , 5–10% MgO). Both the kimberlites and the lamproites are characterized by extremely high abundances of K ($\text{K}_2\text{O} > \text{Al}_2\text{O}_3$, $\text{K}_2\text{O}/\text{Na}_2\text{O} > 10$) and other 'incompatible' elements such as Ba, Rb, Sr, Pb, Th, U, Ti, Zr, Nb and LREE, and together constitute an ultrapotassic suite¹⁰. Detailed descriptions of the geology, petrology, and major and trace element geochemistry are given in refs 10, 11.

The results of the Sm–Nd and Rb–Sr isotopic measurements are listed in Table 1. The measured $^{87}\text{Sr}/^{86}\text{Sr}$ ratios are similar to those reported for the Fitzroy lamproites by Powell and Bell¹³. Initial Nd and Sr ratios calculated using a crystallization age of 20 Myr, are shown in Fig. 1 on a plot of ϵ_{Nd} against initial $^{87}\text{Sr}/^{86}\text{Sr}$. The age correction is insignificant for Nd and, except for the high Rb/Sr samples (WAK-6 and WAK-17), relatively small for Sr. The initial ratios lie to the right of and below the 'bulk Earth', with ϵ_{Nd} values ranging from -7.4 to -15.4 and initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of 0.7104–0.7187. Although the data form a relatively broad array, there is an approximate inverse correlation between samples with more negative ϵ_{Nd} values and high initial $^{87}\text{Sr}/^{86}\text{Sr}$. There is, however, no obvious correlation with geographical locality. For example, kimberlites and lamproites from the northern (Ellendale) area encompass the total range of ϵ_{Nd} values (-7.4 to -15.4). In addition, two intrusive units within a single pipe from Ellendale (WAK-16, WAK-17) have large differences in initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of 0.7104 and 0.7168 respectively. This indicates that the isotopic heterogeneities are on a relatively small scale. There is no obvious distinction between the kimberlites and lamproites although the lamproites have the higher initial Sr ratios. The overlap in isotopic compositions agrees with the petrological and geochemical gradation between the ultrabasic kimberlites and the more felsic lamproites^{10,11} supporting Prider's earlier contention that the Fitzroy lamproites were genetically related to an ultrabasic parent magma resembling kimberlite⁹.

Fig. 1 Nd and Sr initial ratios for kimberlites (◆) and lamproites (●) from the Kimberley region of Western Australia. Fields for mid-ocean-ridge basalts (MORB)^{18,21,25,27} and ocean islands (Azores²⁴, Samoa²⁵, Kerguelen^{25,28}, St Helena²⁵) and Gaussberg³¹, are drawn from previously published data, ◇, South African kimberlites²⁹, and □, diopsides from kimberlite nodules³⁰. With the exception of a kimberlite from Lesotho²⁹, the West Kimberley rocks have significantly more negative ϵ_{Nd} values than previously reported for kimberlites^{1,2}, indicating derivation from ancient (>1,000 Myr) highly enriched sources. Curves show the effects of mixing between depleted mantle (DM) and enriched mantle (EM). Ratios of $(\text{Sr}/\text{Nd})_{\text{DM}}/(\text{Sr}/\text{Nd})_{\text{EM}}$ of from 2 to 10 can account for the dispersion in the West Australian kimberlites and lamproites, as well as the apparently anomalous ocean islands such as Samoa²⁵, Azores²⁴, and St Helena²⁵. The main mantle array has $(\text{Sr}/\text{Nd})_{\text{DM}}/(\text{Sr}/\text{Nd})_{\text{EM}} \sim 5$. The per cent of enriched component is calculated by assuming $(\text{Nd})_{\text{DM}} = 10$ p.p.m. and $(\text{Nd})_{\text{EM}} = 400$ p.p.m.



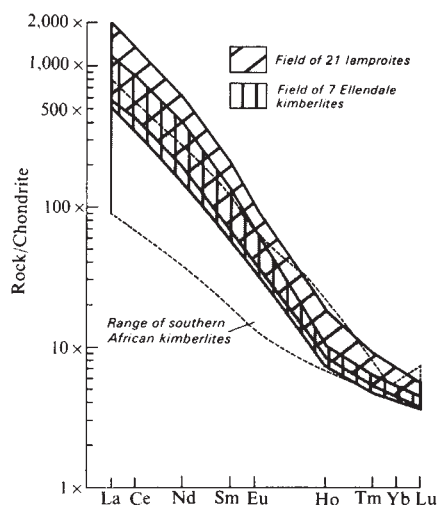


Fig. 2 Chondrite-normalized REE abundances of kimberlites and lamproites from the West Kimberley region of Western Australia. South African kimberlites generally have parallel patterns but lower abundances of REE^{6,40,41}. Contamination with continental crust^{17,42} would be expected to produce significantly lower LREE and higher HREE abundances than are observed in the kimberlites and lamproites.

In view of the distinctly different isotopic compositions of the West Kimberley kimberlites and lamproites, it is important to establish whether they result from melting of enriched mantle or from extensive contamination with continental crust. Several lines of evidence support our contention that the isotopic compositions of the West Kimberley rocks reflect those of their mantle source. First, unlike many of the highly differentiated basaltic to intermediate volcanics for which crustal contamination has been invoked¹⁴⁻¹⁶ the West Kimberley kimberlites and olivine-rich lamproites have clearly been derived directly from the mantle as they contain diamond and other mantle xenocrysts (magnesiocromite, chrome diopside, pyrope). With the exception of WAK 27, all the samples studied have 'primitive' compositions characterized by high Mg/(Mg + Fe) ratios (≥ 0.68) and

high Ni contents (>300 p.p.m.). Second, both the kimberlites and lamproites have similar strongly LREE-enriched REE patterns ($500\text{--}2,000\times$ chondrites) with low abundances of HREE (Fig. 2), and there is a systematic correlation of ϵ_{Nd} with Nd concentration (Fig. 3, inset). This is contrary to that expected for bulk contamination as contamination of a 'pristine' kimberlite magma ($\epsilon_{\text{Nd}} \sim 0$, $\text{Nd} \sim 100\times$ chondrites) with 'typical' Proterozoic continental crust ($\epsilon_{\text{Nd}} \leq -15$, $\leq \text{Nd} \sim 30\times$ chondrites)¹⁷ should result in samples with the most negative ϵ_{Nd} values having the lowest Nd concentration; in fact, the opposite is observed (Fig. 3 inset). Similarly, the lack of positive correlation of $^{87}\text{Sr}/^{86}\text{Sr}$ with either Rb or Sr abundances, together with the high Sr abundances in both the kimberlites and lamproites ($991\text{--}1,904$ p.p.m.), suggests that their radiogenic character is unlikely to result from crustal contamination. Third, in addition to enrichment in Rb, Sr, and LREE (also Ba, Th, U) the West Kimberley rocks have very high abundances of Ti, Zr, and Nb ($2.6\text{--}6.8\%$ TiO_2 , $600\text{--}1,900$ p.p.m. Zr, ≥ 100 p.p.m. Nb) and low abundances of Y (≤ 20 p.p.m.) which are difficult to explain by simple crustal assimilation. Similarly, the low abundances of Al_2O_3 , CaO, and Na_2O ($\leq 9\%$, $\leq 6\%$, and $<1\%$ respectively) seem to preclude extensive assimilation of continental crust of a composition resembling published averages. While the possibility of selective contamination cannot be ruled out special mechanisms are required, such as contamination by Nd-rich phases with negative ϵ_{Nd} (for example, monazite or allanite) and with a highly fractionated REE pattern similar to the kimberlites and lamproites. Preferential enrichment in selected trace elements is also required (for example, Nb relative to Zr and Ti). We therefore consider bulk or selective contamination unlikely and in the following it will be assumed that the measured isotopic characteristics reflect those of the mantle source.

The discovery of highly enriched mantle, as represented by the West Kimberley kimberlites and lamproites, imposes an additional important constraint on mantle evolution models. Of particular relevance is the origin of the approximate inverse correlation between $^{87}\text{Sr}/^{86}\text{Sr}$ and $^{143}\text{Nd}/^{144}\text{Nd}$ ratios (the mantle array). At present, there is no consensus on the origin of this relationship, with explanations including mixing of depleted mantle with undepleted^{18,19} or enriched mantle reservoirs²⁰, or continual magmatic depletion of a planetary

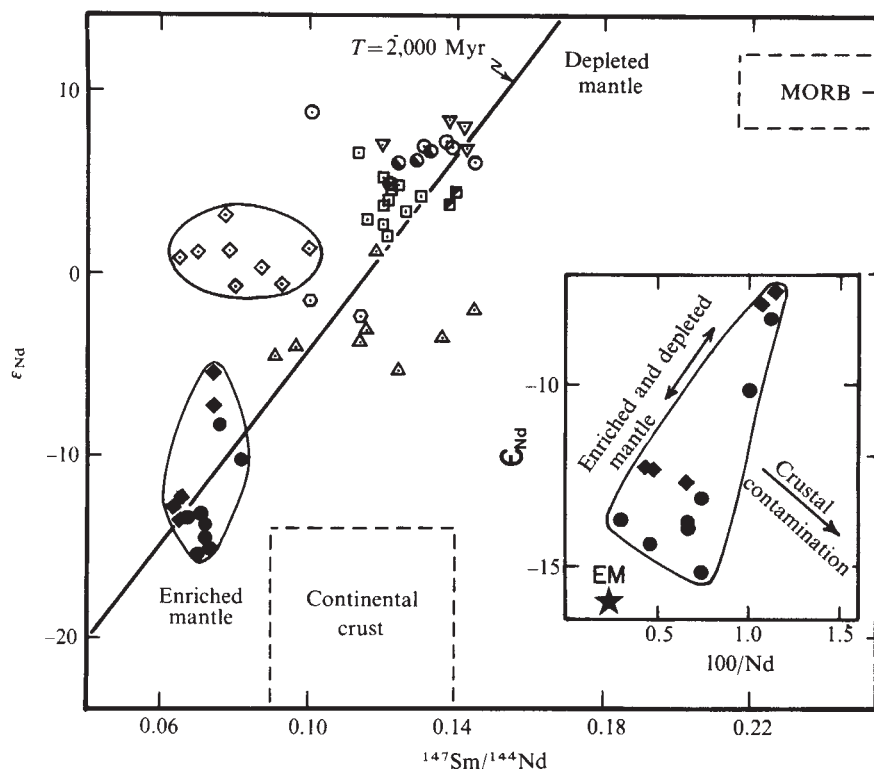


Fig. 3 ϵ_{Nd} plotted against $^{147}\text{Sm}/^{144}\text{Nd}$ and $100/\text{Nd}$ (inset) for kimberlites (♦) and lamproites (●) from the Kimberley area of Western Australia. Kimberlites from other localities (◇)^{1,2}. Ocean island alkali basalts: ○, Hawaii²⁷; △, Kerguelen²⁸; □, Azores²⁴; ⊙, Tristan da Cunha²⁷; ▽, Ascension²⁷; ■, Bouvet²⁷; and ⊖, Iceland²⁷. Kimberlites and lamproites from Western Australia show an approximate linear correlation between ϵ_{Nd} and $100/\text{Nd}$ which is also consistent with mixtures of enriched and depleted mantle reservoirs but is opposite to the trend expected for bulk crustal contamination. The time scales for the formation of the enriched and depleted reservoirs are $>1,000$ Myr and more than two components seem to be involved.

reservoir^{21,22}. Recently, Hofmann and White²³ have proposed an additional model involving recycling of crustal material into the mantle. We propose a mixing model in which highly enriched mantle similar to that identified here is mixed with depleted (MORB-type) mantle. This model can, in principle, provide an explanation of both the main mantle array as well as the apparently disparate data for the Azores²⁴, Samoa²⁵, Society²⁵, St Helena²⁵ and Tabar To Feni²⁶ ocean islands. This is illustrated in Fig. 1, where mixing lines are shown between a depleted mantle component (DM) and the highly enriched mantle (EM). Due to the relatively broad field defined by the West Australian kimberlites and lamproites, a continuum of mixing lines is required with $(\text{Sr}/\text{Nd})_{\text{DM}}/(\text{Sr}/\text{Nd})_{\text{EM}} \sim 2-10$. Thus, mixing between enriched mantle and depleted mantle with $(\text{Sr}/\text{Nd})_{\text{DM}}/(\text{Sr}/\text{Nd})_{\text{EM}} \sim 4-5$ accounts for the main mantle array, while mixtures with $(\text{Sr}/\text{Nd})_{\text{DM}}/(\text{Sr}/\text{Nd})_{\text{EM}} \sim 10$ and ~ 2 can account for the remaining apparently deviant samples. The proportions of enriched mantle in a particular sample can be estimated if we assume Nd or Sr concentrations in the endmember components. For example, with $(\text{Nd})_{\text{EM}} = 400$ p.p.m. and $(\text{Nd})_{\text{DM}} = 10$ p.p.m., a sample with $\epsilon_{\text{Nd}} = 0$ requires the addition of only $\sim 2\%$ of enriched component. Nevertheless, this relatively small proportion of enriched component will still dominate the abundances of the 'incompatible' elements Ba, Rb, Sr, Pb, Th, U, Ti, Zr, Nb and LREE because of their extremely high abundances in the enriched mantle endmember.

This general type of mantle mixing model has previously been discussed by Anderson²⁰ but in his model the depleted and enriched reservoirs were complementary, such that $(\text{Sr}/\text{Nd})_{\text{DM}}/(\text{Sr}/\text{Nd})_{\text{EM}} \sim 1$. A feature of these mixing models is that rocks with positive ϵ_{Nd} (indicating an integrated history of Nd/Sm depletion) can have LREE enrichments (Nd/Sm ratios greater than chondritic). This is illustrated in Fig. 3 in a plot of ϵ_{Nd} against $^{147}\text{Sm}/^{144}\text{Nd}$, on which simple mixes will produce straight lines between endmembers. The oceanic-island alkali basalts^{24,27,28} are generally consistent with this type of model, although the range of $^{147}\text{Sm}/^{144}\text{Nd}$ ratios indicates fractionation of the LREE as well as mixing between endmember components.

The field of kimberlites from southern Africa, India, the United States, and Soviet Union^{1,2} is also shown in Fig. 3. With the exception of a Lesotho kimberlite from Kao mine which has an $\epsilon_{\text{Nd}} = -11.0$ (ref. 29) comparable with the West Kimberley rocks studied here, most of these kimberlites cluster near $\epsilon_{\text{Nd}} \sim 0$ (+3.6 to -0.6). These rocks can also be modelled as blends of enriched and depleted mantle; the clustering of ϵ_{Nd} values near zero being entirely fortuitous and simply reflecting approximately constant proportions of enriched ($\sim 2\%$) and depleted components. An origin by mixing of enriched and depleted mantle components for these rocks is in agreement with the model proposed by Nixon *et al.*⁷ on the basis of the major and trace element chemistry of the kimberlites and their inclusions.

The isotopic composition of the enriched mantle component is arbitrary, being simply constrained by the most negative ϵ_{Nd} value and the highest $^{87}\text{Sr}/^{86}\text{Sr}$ ratio. Several enriched mantle components are possible. For example, clinopyroxenes from southern African kimberlites^{30,34} and leucite-bearing lavas from Gausberg³¹ suggest an enriched mantle with similar ϵ_{Nd} values (-12 to -14) but with lower $^{87}\text{Sr}/^{86}\text{Sr}$ ratios (≤ 0.710). In addition, Pb isotopic data clearly require at least three mantle components in addition to the continental crust³². Nevertheless, the interaction of enriched mantle components with depleted parts of the mantle as implied by this study seems to be an important process.

The origin of ancient enriched mantle, now reported from both oceanic^{25,28,33} and continental areas^{30,31,34}, is controversial, and has been ascribed to metasomatic processes³⁵, addition of an enriched component from melting of ancient subducted oceanic²³ or continental crust³⁶, or the existence of a primary global enriched reservoir²⁰. Enrichment of the mantle in 'incompatible' elements by metasomatic processes has commonly been

invoked in the petrogenesis of alkaline basaltic rocks³⁷ and it is widely believed that the mantle beneath many, if not most, areas of alkaline magmatism has been metasomatized resulting in chemical heterogeneities that may have persisted for long periods of time^{35,38}. A similar model involving addition of an 'incompatible' element-enriched fluid phase (H_2O - and F-rich) to depleted mantle (diopside-poor garnet lherzolite or garnet harzburgite) has been suggested by Jaques *et al.*¹⁰ to account for the depleted chemistry of the xenocryst and xenolith suites and the low abundances of lithophile elements such as Al, Ca, and Na in the West Kimberley rocks. The refractory peridotite xenoliths may represent mantle residual from earlier (Proterozoic) magmatic episodes. The highly enriched mantle identified in this study requires extreme LREE (Nd/Sm) and Rb/Sr enrichments for relatively long time scales. For example, using a depleted mantle model the samples give calculated $T_{\text{DM}}^{\text{Nd}}$ ages ranging from 920 to 1,310 Myr (Table 1). This represents the time required for the West Kimberley rocks to evolve from initially depleted mantle source ratios (that is positive ϵ_{Nd}) to their observed negative ϵ_{Nd} values with their measured Sm/Nd ratios. These ages must be considered minimum ages as the samples have extreme LREE enrichment; with more realistic estimates of the Sm/Nd ratios in the source the time scales would be increased considerably (Archaean). Thus, extremely ancient enrichment events are required. The origin of the enriched mantle remains uncertain since none of the models proposed to date, including those invoking recycling of crust, are unique and all must be regarded as speculative.

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Analysis and interpretation of Holocene sea-level data

Ian Shennan, Michael J. Tooley, Martin J. Davis
& B. Andrew Haggart*

Department of Geography, University of Durham,
Science Laboratories, South Road, Durham DH1 3LE, UK

The geography of the geoid¹ and the lack of an acceptable methodology and definition of terms for sea-level studies have impeded the realization of the objectives of the International Geological Correlation Programme Project 61 on Holocene Sea-Level Changes²; these were to establish a graph of the trend of mean sea level over the globe and the relationship between sea level, climate change and the global ice budget². A solution to the problems of correlation is proposed here. The use of terms 'transgression' and 'regression' has been a major cause of misinterpretation³ and the terms 'transgressive overlap' and 'regressive overlap' have been proposed as descriptive lithostratigraphic terms, in which no process is implied³⁻⁵. The processes involved in the development of coastal stratigraphical sequences are dependent on the position and rate of change of sea level, and these sequences contain evidence of tendencies of sea level⁶⁻⁸. The application of the concept of sea-level tendency permits meaningful correlations between rising and subsiding areas, and introduces an objectivity to correlation schemes showing 'transgression sequences'^{9,10}.

Changes in sea level affect low-lying coastal areas, and a rise or fall in sea level will be registered by changes in vegetation and lithology, the precise nature and character of which are local and site-dependent. The dominant direction of change will be constant and recorded over a much wider area, the expression of which is defined as a tendency of sea-level movement⁸. Detailed interpretation of regional phenomena cannot be made from an evaluation only of sea-level index points on a time-altitude diagram, because the errors involved in the estimation of past altitudes permit only the identification of a broad sea-level band. Therefore, it is difficult to evaluate the accuracy of chronological correlation schemes based on the comparison of sea-level curves.

Correlation schemes based on the sea-level tendency concept offer an alternative approach, and the method has already been shown to be adequate for the Fenland and north-west England data². The Fenland chronology has been developed from 106 ¹⁴C dates. Of these, 47 (Fig. 1a) can be used as the basis for the chronology of positive and negative tendencies of sea-level movement (Fig. 1b), with lithostratigraphical information used to confirm the tendencies^{4,11}. The scheme is presumed to be accurate to about ± 100 ¹⁴C yr. Figure 1c shows a method by which the dated sea-level index points can be used to develop a first stage chronology where detailed knowledge of the stratigraphical sequences is not available. The combined frequency histograms produce a very similar chronology³.

From 74 ¹⁴C dates from north-west England, 70 are from transgressive or regressive overlaps (Fig. 2a) and 4 refer to positive or negative sea-level tendencies inferred from periods of sand dune stability or migration (Fig. 2b). These have been subjected to histogram analysis. Two further periods of transgressive overlap have been established stratigraphically for which there is no direct ¹⁴C evidence. These data have been amalgamated into a partial chronology of sea-level tendencies (Fig. 2d). Time limits for each period are set by the means of the oldest and youngest radiocarbon dates. Subsequent analysis to produce a continuous chronology will be to estimate a common boundary for each positive/negative tendency period by

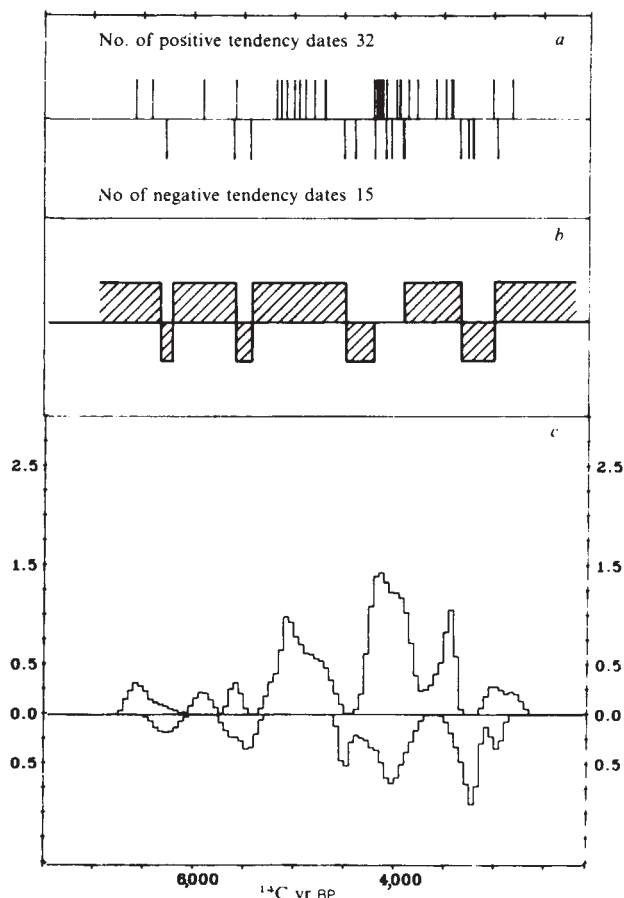


Fig. 1 a, Distribution of 47 ¹⁴C dates from the Fenland which are related to positive or negative tendencies of sea-level movement. b, Continuous chronology of tendencies of sea-level movement derived from all ¹⁴C and stratigraphical data available. c, Combined frequency histograms of the 47 dates; the y-axis represents a probability scale for dates occurring within a certain 50-yr interval given the distribution of ¹⁴C data in a.

applying the histogram technique or by comparing the proposed chronology with the detailed stratigraphical evidence.

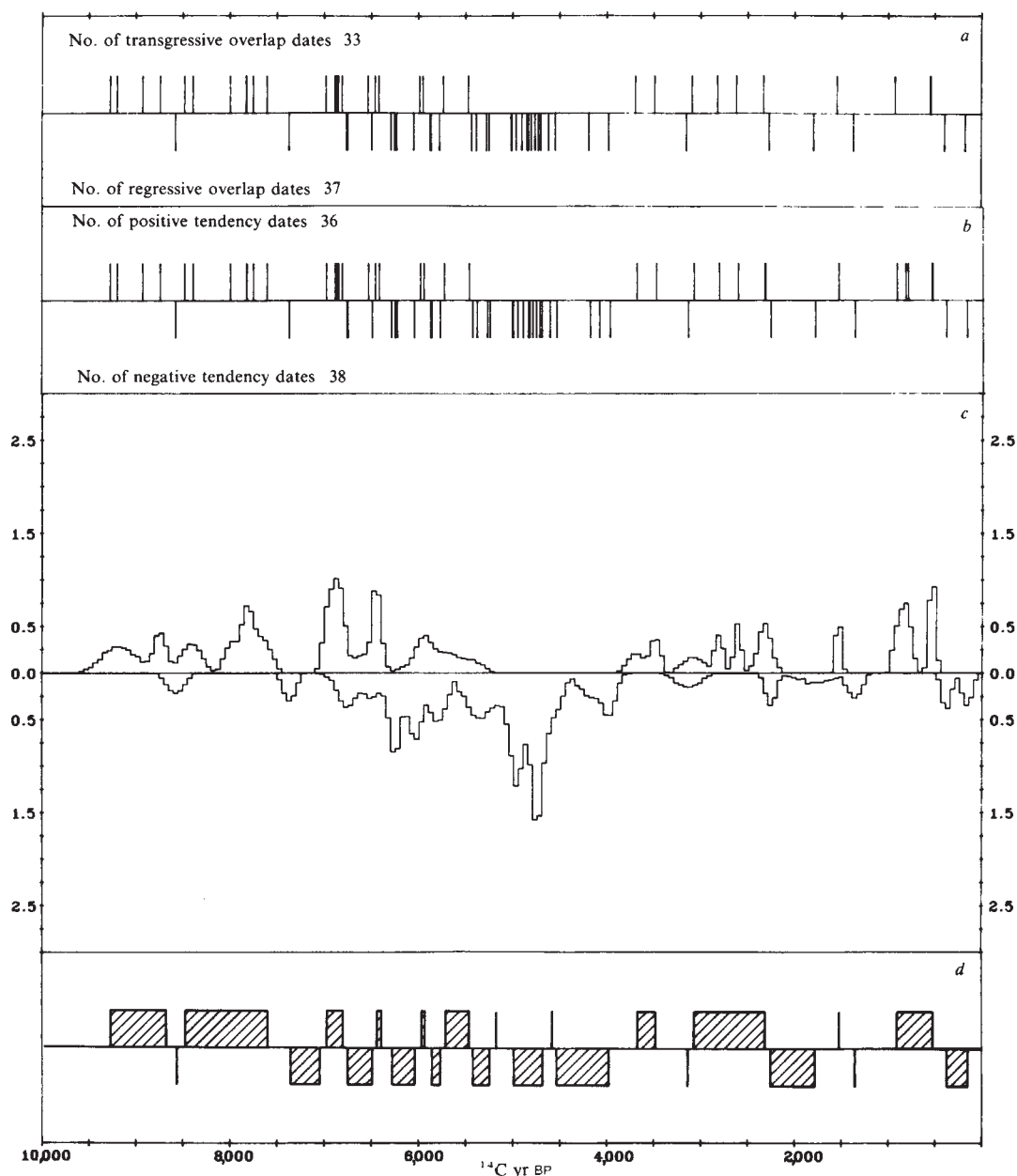
The Tay Estuary area is the subject of a detailed and securely dated published record of sea-level change in Scotland. A total of 37 ¹⁴C dates and detailed morphological, stratigraphical and micropalaeontological evidence are available¹²⁻¹⁵. Of these, 32 dates may be reliably related to tendencies of sea-level movement. Figure 3a shows 29 dates on transgressive or regressive overlaps. Three dates are excluded because there is a significant hiatus between the deposition of marine-estuarine sediment and subsequent peat growth, which means that they are not regressive overlaps as defined by Tooley⁵. Indeed, the peat growth is associated with a subsequent rise in water table consequent on a rising sea level^{13,15} and so records positive tendencies (Fig. 3b). Figure 3a and 3b differ further in that two dates on regressive overlaps are interpreted as positive tendencies, as the subjacent grey sand layer is believed to represent a storm surge before the culmination of regional positive tendencies¹⁶.

The histogram analysis (Fig. 3c) only indicates a discrepancy at ~6,700 BP. The negative tendency peak is from a single date at Fullerton (SSR. 1148, 6704 $\pm$ 55 BP), which is considerably older than those obtained in the Tay area. The stratigraphical section¹⁵ indicates a regressive overlap but with the marine-estuarine sediments continuing to be deposited a further 1.5 m higher. Local peat growth has been shown to keep pace with sea-level rise in other locations^{17,18} and the date may represent a positive tendency of sea-level movements.

A partial chronology is derived from this analysis (Fig. 4d). The first two periods of negative tendencies could have been

* Present address: Geography Section, City of London Polytechnic, Calcutta House Precinct, Old Castle Street, London E1 7NT, UK.

Fig. 2 *a*, Distribution of 70 ^{14}C dates on transgressive or regressive overlaps from north-west England. *b*, Distribution of 74 ^{14}C dates related to positive or negative tendencies of sea-level movement. *c*, Combined frequency histograms of the 74 dates. *d*, Partial-chronology of tendencies of sea-level movement in north-west England.



shown as one but there is a lack of ^{14}C data. However, Cullingford *et al.*¹³ indicate a period of positive tendencies at that time.

Thus, the application of a uniform method of data collection, analysis and evaluation allows a comparison of the chronologies from the Fenland, north-west England and the Tay area. In an uplifted area it is probable that only positive tendencies of sea-level movement should be of more than local significance because negative tendencies may be a result of isostatic and sea-level movements. In subsiding zones only negative tendencies of sea-level movement will be of more than local significance because positive tendencies may be the result of subsidence.

This concept has been tested using the Fenland and north-west England data⁴. The cross-association χ^2 test can be used to assess the degree of matching between two sequences¹⁹. When the two original chronologies, for the period 6,700–2,500 BP (Fig. 4a), are compared at 50-yr intervals, the observed number of matches between the two successions is no greater than that expected from two random sequences of equivalent composition. This is not unexpected because the same result was obtained from a series comparing hypothesized uplifted and subsiding zones³. However, when only the periods during which either the Fenland chronology indicates negative tendencies or the north-west England chronology indicates

positive tendencies are compared (Fig. 4b), the number of matches is greater than that expected from two random sequences (at 1% significance level).

Similar analyses have been carried out for the partial chronologies of sea-level tendencies from north-west England and the Tay area (Fig. 4a, c). In this case, north-west England is a subsiding zone relative to the Tay area and therefore the chronologies can be used to reveal non-local phenomena when the north-west England scheme shows negative tendencies and when the Tay scheme shows positive tendencies. There is perfect agreement except for two periods.

At 6,750 yr BP there is disagreement but this lies within the dating errors and arises from the need to establish the age of the change from positive to negative tendencies at Fullerton, discussed above. The greatest disagreement occurs between the two chronologies at 7,400–7,150 yr BP. As the Tay data reveal incontrovertible evidence for positive tendencies, the evidence for a period of negative tendencies in north-west England requires scrutiny. Here the period is defined from two assays, one from Lough Cranstal on the Isle of Man and the other from Bootle Beach in Tarn Bay, Cumbria. At Lough Cranstal^{18,19}, positive tendencies at $7,825 \pm 120$ yr BP are indicated by a change in lithology, and by brackish water and marine micro- and macrofossils. These are soon extinguished within

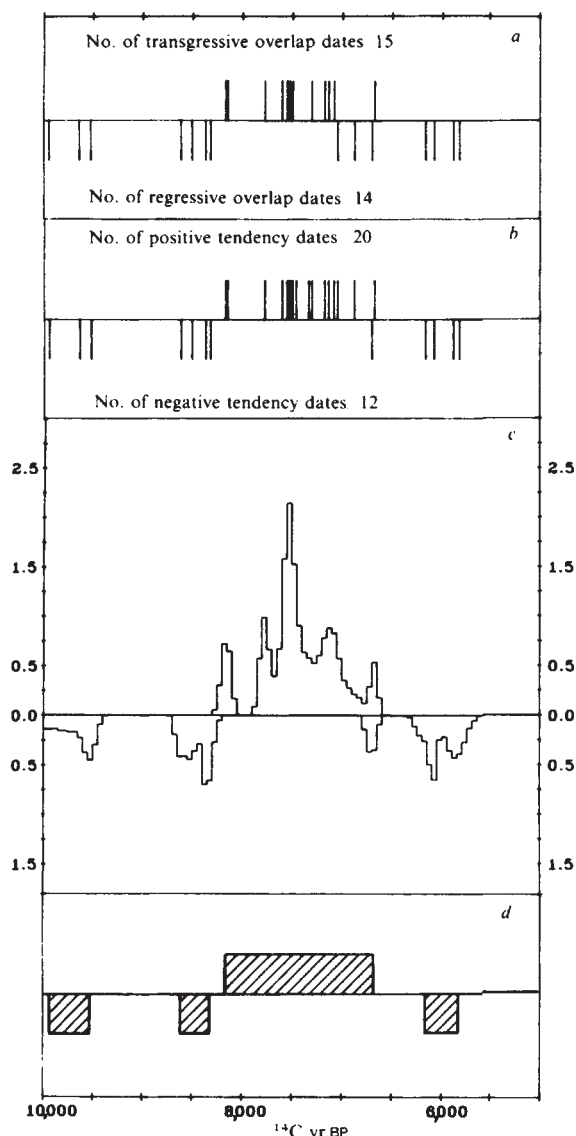


Fig. 3 *a*, Distribution of 29 ^{14}C dates on transgressive or regressive overlaps from the Tay Estuary area. *b*, Distribution of 32 ^{14}C dates interpreted as positive or negative tendencies or sea-level movements. *c*, Combined frequency histograms of the 32 dates. *d*, Derived partial chronology of tendencies of sea-level movement in the Tay Estuary area.

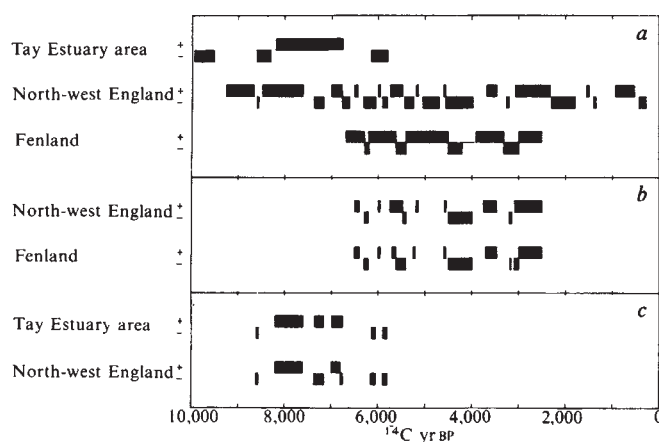


Fig. 4 *a*, Chronologies of tendencies of sea-level movement from the Tay Estuary area, north-west England and the Fenlands. *b*, Partial chronologies from north-west England and the Fenland for tendencies of more than local significance. *c*, Partial chronologies from the Tay Estuary area and north-west England for tendencies of more than local significance.

the clastic bed and replaced by freshwater indicators—fruits of *Ceratophyllum* and *Potamogeton*, and diatoms such as *Fragilaria construens*, *Cymbella leptoceros*, *Stauroneis anceps* and *Eunotia* spp.^{20,21} at $7,370 \pm 110$ yr BP. This premature extinction was caused by local conditions: the lough never had an open water connection with the sea, but was separated from it by a narrow valley at the mouth of which was a shingle beach.

At Bootle Beach^{20,22}, the stratigraphy shows a clastic bed intercalating organic deposits, and is interpreted as evidence of a positive sea-level tendency succeeded by a negative sea-level tendency $7,160 \pm 75$ BP. However, the clay bed contains the seeds and fruits of *Alnus glutinosa*, *Ranunculus* subgenus *Batrachium*, *Betula* cf. *pubescens*, *Potamogeton perfoliatus* and *P. obtusifolius*, as well as *Daphnia ephippia* and a stratoblast of *Cristatella mucedo*, all of which are freshwater indicators.

Re-examination of the evidence from these sites allows re-interpretation and permits closer correlations between the data sets from the Tay area and north-west England.

The exploratory approach outlined here is consistent, flexible, easy to apply and has given good results in the situations to which it has been applied. However, it is only a first stage of analysis to resolve the relationship between sea level, ice volume, climate and problems of recent crustal movements. These correlations and relationships have been elusive but the new method based on the sea-level tendency concept will permit meaningful correlations of sea-level chronologies, and provide a proxy record of climatic and ice volume change.

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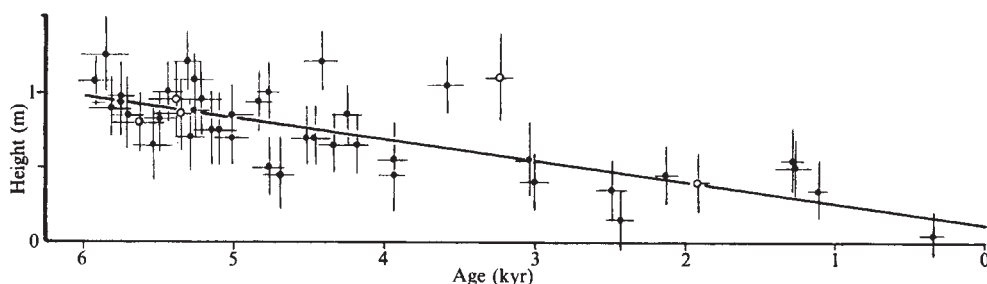
Evidence for smoothly falling sea level relative to north Queensland, Australia, during the past 6,000 yr

John Chappell

Biogeography and Geomorphology, Research School of Pacific Studies, Australian National University, PO Box 4, Canberra, ACT 2600, Australia

Post-glacial sea-level curves are expected to differ between different parts of the world, as a result of global isostatic changes¹ or perhaps other forms of geoidal changes². I report here results from the inner part of the northern Great Barrier Reef which seem to show a pattern of smoothly falling sea level

Fig. 1 Age-height plot of north Queensland microatoll sea-level data. Vertical bars span maximum and minimum estimates of past mean low water spring tide level (MLWS) relative to present MLWS, for each station. Horizontal bars span $\pm 1\sigma$ for radiocarbon age error. Mean values are shown as circles. Solid circles, data from Table 1; open circles, data from McLean *et al.*⁴ with ages normalized as for Table 1 results, and vertical error ranges estimated by this author. From McLean *et al.* these results are: Low Wooded, 0.6–1.0 m at $5,630 \pm 95$ yr BP; Houghton, 0.7–1.2 m at $5,400 \pm 175$ yr BP; Leggatt, 0.6–1.1 m at $5,350 \pm 153$ yr BP; Nymph, 0.8–1.4 m at $3,250 \pm 95$ yr BP; E. Petherbridge, 0.2–0.6 m at $1,920 \pm 80$ yr BP. The solid line is best-fit least-squares regression.



during the past 6,000 yr. The results are consistent with stratigraphical results from prograded mangrove coasts on the nearby north Queensland mainland. The region is remote from the tectonically active margin of the south-west Pacific Ocean, and the results contribute to the global data set relevant to isostatic changes being assembled by the International Sea Level project (IGCP Project No. 61). They also provide a baseline relevant to Holocene coral reef growth in the inner Great Barrier Reef.

The new north Queensland sea-level evidence comes from fringing coral reefs which abut on bedrock outcrops at islands near the mainland coast. Fringing reef flats at these islands range from 200 to 1,000 m wide and slope gently seawards. The flats have a thin veneer of muddy sediment and coral detritus. In places, low banks of coral shingle, sometimes cemented to some extent, occur on the flats. For purposes of identifying past sea levels, attention was focused on coral microatolls. The microatoll form, and its occurrence in the Great Barrier Reef, is reviewed by Scoffin and Stoddart³ who, with McLean *et al.*⁴, show that microatolls growing in open water occur up to levels close to mean low water spring tide (MLWS). In ponded or shallow lagoonal situations the mean lowest water level can stand significantly above MLWS, and in such sites the microatolls grow correspondingly higher^{3,4}. In this study, care was taken to exclude any groups of ancient microatolls where the facies of the reef flats, inspected where necessary by digging, suggested that ramparts or shingle banks may have induced ponding. Although the possibility of ponding cannot be entirely excluded, consistency among the results suggests that the problem has been avoided.

The island sites occur between Stone Island at 20° S and King Island at 14° S, and lie within about 25 km of the mainland coast. Thom and Chappell⁵ suggested that hydro-isostatic warping affected the Great Barrier Reef shelf during and after post-glacial sea-level rise. Chappell *et al.*⁶ show that sea-level indicators from a wide range of north Queensland coastal and reef sites, dated to ~5,500 BP, fit quite closely to a computed model of hydro-isostatic warping. In order that results discussed here should be comparable, relative to datum, all sites were chosen to lie as close as possible to the +1 m, 5,500 BP isobase deduced by Chappell *et al.*⁶. All sample sites contained groves of ancient microatolls, usually with other *in situ* coral remains, and all were judged to have been palaeoecologically rather similar.

Sites and summary results are listed in Table 1. At each reef flat site, one or more traverses were levelled to benchmarks provided in most cases by the Australian Survey Office. These were tied to tidal datum. Each observation in Table 1 represents a group of microatolls, constituting a station on a traverse. Several microatolls were levelled at each station, and several living microatolls were levelled at the seaward end of each traverse. Samples for radiocarbon dating were taken at each station. Dated samples were 100% aragonite, pretreated with chlorox and dilute HCl washes, plus ultrasonic cleaning. Ages were measured by the Australian National University Radiocarbon Laboratory and by Beta Analytic (Miami), with cross-checks between the two. Ages in Table 1 are normalized for isotopic fractionation⁷, and have been put on the same basis as

terrestrial samples by subtracting the Gillespie–Polach⁸ correction factor for eastern Australian coastal waters of 450 ± 35 yr.

It was found that oldest ages and highest elevations occur at the inner margin of the fringing reefs at all sites, and that microatoll groups become lower and younger towards the seaward reef margins, suggesting outgrowth during falling sea level. To estimate relative sea-level change, the height variation of microatolls at each site is calculated as follows. Let the minimum height of living microatolls at a traverse = a and their maximum = b ; let the minimum height among a group of dead microatolls at a station = A and their maximum = B . The minimum change in MLWS = $A - b$, and the maximum change = $B - a$. The maximum and minimum values in Table 1 were established in this way, and represent the range of estimated change of sea level between formation of the microatolls at a station and the present time. The results assume that environmental conditions for the ancient groups of microatolls were similar to those growing today at the reef margins.

Table 1 Changes of mean low water spring tide level and age data from north Queensland inner shelf islands

Δ MLWS (m)				Δ MLWS (m)			
Max.	Min.	Age	$\pm$ Error	Max.	Min.	Age	$\pm$ Error
Orpheus 146°29' E; 18°36' S				Fantome 146°32' E; 18°40' S			
1.15	0.75	4,860	90	1.2	0.8	5,460	95
0.85	0.45	4,190	95	1.1	0.7	5,280	110
0.6	0.2	3,030	80	0.85	0.45	4,350	90
0.35	-0.05	2,460	80	0.65	0.25	2,160	90
Great Palm 146°36' E; 18°42' S				0.55	0.15	2,550	90
1.0	0.6	5,490	115	Goold 146°10' E; 18°52' S			
1.05	0.65	5,020	105	1.5	1.0	5,850	145
1.05	0.65	4,260	95	1.2	0.7	5,215	115
Dunk 146°10' E; 17°57' S				0.8	0.3	3,050	80
1.1	0.6	5,725	105	Yule* 145°30' E; 16°35' S			
0.95	0.45	5,295	95	1.4	1.0	4,420	100
0.7	0.2	4,695	100	0.9	0.5	4,475	125
Magnetic 146°52' E; 19°09' S				1.25	0.85	3,600	95
1.4	1.0	5,325	80	0.75	0.35	1,300	70
1.2	0.8	4,770	80	0.55	0.15	1,150	65
0.7	0.3	4,765	115	Camp 147°53' E; 19°52' S			
0.9	0.5	4,530	105	1.1	0.7	5,820	115
0.2	-0.1	360	70	0.9	0.5	5,020	100
Stone 148°16' E; 20°02' S				0.7	0.3	1,280	100
1.25	0.9	5,925	115	Flinders 144°14' E; 14°11' S			
1.15	0.8	5,755	105	1.2	0.7	5,750	200
1.25	0.9	5,285	135	0.9	0.4	5,550	105
King 144°19' E; 14°05' S							
1.0	0.5	5,150	105				
1.0	0.5	5,100	105				
0.8	0.3	3,950	95				
0.7	0.2	3,950	95				

¹⁴C ages normalized for isotopic fractionation and with seawater correction subtracted (see text).

* Mainland coastal site, also dated by Bird¹⁹.

Age-height data from Table 1 are plotted in Fig. 1, as are results published by McLean *et al.*⁴ from five other islands which lie close to the 5,500 yr BP +1 m isobase (see Fig. 1 legend). A minimum hypothesis is that these data fall on a straight line. Figure 1 shows the least-squares linear regression, $H = 1.36 \times 10^{-4}T + 0.16$, where H is height of MLWS relative to present, in metres, and T is yr BP. Total r.m.s. error is ± 0.2 m in the vertical sense. Very few points lie off the line without conflicting data of the same age on or near the line. Two outliers are Yule 3,600 yr BP and Nymph 3,250 yr BP. Yule lies about 25 km landwards of the 5,500 yr BP +1 m isobase, and Nymph was not visited in this study but is a result from McLean *et al.*⁴. I conclude that the straight line is the simplest model fitting these results.

The smoothly falling sea level suggested by the straight line may not be the only interpretation, in the light of careful work performed elsewhere in the world. A strong case has been made for alternating transgressive and regressive tendencies of sea level of amplitudes around 1 m or more, superimposed on the general post-glacial trend in each region of interest, for example by Tooley^{9,10} from many studies in the United Kingdom, by Morner¹¹ from Sweden, by Martin *et al.*¹² from South American sites, and from Oceania by Schofield¹³. Despite the finding that the microatoll results conform to the straight line in Fig. 1, two factors suggest that sea-level oscillations may not be recorded by this form of evidence. (1) Evidence for regressions may be buried within the reef flats by subsequent transgressional effects. (2) Regression phases may be sandwiched in the gaps between the dating results. For example, the two outliers above the line in Fig. 1, at 3,600 BP, may correspond with the Calais IV-B transgression in the Netherlands¹⁴, although their radiocarbon ages are rather younger. Note that the Netherlands results show fluctuations of ~ 0.5 m about the mean trend¹⁴, which may not be visible in the microatoll sequences.

A second set of evidence comes from broad prograded mud and shell-chenier plains on the mainland Queensland coast, occurring at Broad Sound¹⁵, Cleveland Bay¹⁶ and Princess Charlotte Bay¹⁷ on the Great Barrier Reef coast, and at Karumba in the Gulf of Carpentaria¹⁸. In each case, on the basis of stratigraphical coring or trenching and radiocarbon dating studies, progradation since about 6,000 yr BP is inferred in conditions of falling sea level by the respective authors. The amount of sea-level fall differs between these sites in a pattern which seems to accord with expected post-glacial hydro-isostatic warping⁶. No evidence indicative of transgressive/regressive cycles, superimposed on the general sea-level trends, has yet been recorded at these sites.

In conclusion, sea level relative to the inner margin of the Great Barrier Reef seems to have followed a smoothly falling trend in the past 6,000 yr. Evidence for sea-level oscillations of greater than 1 m, identified elsewhere in the world, has not been found, despite the fact that results given here from fringing coral reefs, and by others from prograded coastal plains in northern Australia, are now relatively numerous. Differences in general trend, between different regions, are likely to reflect effects of postglacial global isostasy. It is now important to resolve the question of lesser oscillations, or transgressive/regressive cycles. If the apparent contrast between these north Queensland results and those elsewhere is real, other geophysical phenomena must be implicated.

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Uranium-series dating of molluscs from uplifted Holocene beaches in the Persian Gulf

M. Ivanovich*, C. Vita-Finzi† & G. J. Hennig‡

* Nuclear Physics Division, AERE, Harwell OX11 0RA, UK

† University College London, Gower Street, London WC1E 6BT, UK

‡ Niedersächsisches Landesamt für Bodenforschung, Postfach 510353, 3000 Hanover 51, FRG

The study of coastal deformation and sea-level history calls for radiometric ages on shorelines older than the past 20,000 yr, the period for which reliable ^{14}C ages can be obtained. Uranium-series disequilibrium methods have yielded the requisite ages for reef coral ranging from 5,000 to 300,000 yr but previously have proved unreliable for most molluscs, the major source of uncertainty being postmortem migration of uranium and its daughter radionuclides into and out of the shells. In the present study the $^{230}\text{Th}/^{234}\text{U}$ dating method¹ was applied to molluscs from Holocene beaches in the Persian Gulf which had been removed from marine influence by seismic uplift. Two specimens selected for comparative dating with the electron spin resonance (ESR) method yielded ages of the correct order of magnitude. Excellent agreement was obtained between ^{14}C and $^{230}\text{Th}/^{234}\text{U}$ ages after detrital effects were corrected for by reference to modern comparative material for both calcitic and aragonitic specimens, which suggests that uranium-series dating of pre-Holocene molluscs may give reliable results provided the depositional history of the shoreline is well documented.

The classic studies of uranium-disequilibrium dating of marine molluscs by Broecker, Kaufman and others^{2–4} might have been expected to deter any geologist from applying the method. For, quite apart from any disagreement between uranium-series and ^{14}C ages, which could always stem from errors in the radiocarbon dates^{5,6}, the evidence they present points to the migration of uranium isotopes into or out of the shell after the death of the mollusc. Modern mollusc shells generally contain <0.5 p.p.m. of uranium, and fossil shells average 1 p.p.m. regardless of age^{2,3}. Furthermore, the $^{234}\text{U}/^{238}\text{U}$ activity ratios in modern marine shells are close to 1.15, the accepted value for oceanic waters³, whereas the uranium isotope activity ratios in fossil shells are commonly greater than 1.15 indicating assimilation and uptake of uranium isotopes at least partly from sources other than oceanic waters^{4,7}. It has been suggested that uranium uptake occurs for a few thousand years² and perhaps as much as 10,000 yr after death. The addition of other radionuclides such as ^{230}Th , ^{231}Pa and ^{226}Ra is also suspected^{2,4}. The fact that uranium-series ages sometimes agree with other dating methods or are at least consistent with the stratigraphical order of their host deposits is thus of little comfort when it comes to dating specimens for which no cross-checks are available.

Table 1 Uranium series disequilibrium obtained from molluscs from uplifted beaches in Persian Gulf

Site	Sample no.	Species*	Uranium content (p.p.m.)†	Activity ratios				$^{230}\text{Th}/^{234}\text{U}$ age (yr BP)		^{14}C age § (yr BP)	^{14}C age calibrated ^{20,21} (yr BP)	Laboratory no. for ^{14}C measurement
				$^{234}\text{U}/^{238}\text{U}$	$^{230}\text{Th}/^{232}\text{Th}$	Uncor-rected	Cor-rected‡	Uncor-rected	Cor-rected‡			
Tujak (Iran)	ETIb	<i>Crassostrea cf. cucullata</i>	0.12	1.294	2.4	0.070	0.018	7,900	2,000	1,700	1,790–1,470	QC-549
	HAR 2223		±0.01	±0.136		±0.015	±0.004	±1,800	±500	±105		
	ETIIo		0.03	1.164	2.1	0.202	0.035	24,400	3,900	2,330	2,545–2,155	SRR-1313
	HAR 2224		±0.01	±0.264		±0.081	±0.014	±11,400	±1,800	±50		
	ETIa	<i>Scapharca inaequivalvis</i>	0.44	1.179	4.6	0.039	0.024	4,400	2,600	1,530	1,455–1,365	SRR-1258
	HAR 2231		±0.02	±0.053		±0.006	±0.004	±700	±400	±45		
	ETIIb		0.15	1.282	13.0	0.113	0.099	13,000	11,300	3,325		
	HAR 1701		±0.01	±0.098		±0.022	±0.019	±2,700	±2,400	±85	3,830–3,360	UM-1260
	ETIIb		0.19	1.241	15.0	0.288	0.262	36,400	32,700	6,110	8,095–7,345	UM-1269
	HAR 2233		±0.01	±0.087		±0.039	±0.035	±5,900	±5,000	±95		
	ETIc	<i>Asaphis deflorata</i>	0.11	1.576	3.0	0.074	0.031	8,300	3,400	2,910	3,290–2,840	SRR-1309
	HAR 2227		±0.01	±0.155		±0.013	±0.005	±1,600	±700	±50		
	ETIIx		0.16	1.257	3.7	0.106	0.057	12,100	6,400	6,170		
	HAR 2228		±0.01	±0.112		±0.016	±0.009	±1,900	±1,000	±155	7,330–6,705	UM-1255
Khor (Qatar)	QB 19	<i>Asaphis deflorata</i>	0.12	1.098	2.6	0.112	0.037	12,900	4,100	4,690	5,720–5,115	HAR-523
	HAR 2229		±0.01	±0.079		±0.016	±0.005	±2,000	±600	±80		
Rud-e Chaharu (Iran)	EJ2	<i>Oliva bulbosa</i>	0.20	1.256	2.3	0.179	0.045	21,300	5,000	4,870	5,835–5,325	UM-1151
	HAR 1708		±0.01	±0.117		±0.028	±0.007	±3,700	±900	±100		
Sadaich (Iran)	1A1s	<i>Amiantis hagenowi</i>	0.07	1.542	3.9	0.107	0.061	12,200	6,800	7,300	8,555–7,635*	MC-1098
	HAR 1618		±0.01	±0.133		±0.026	±0.015	±3,100	±1,700	±140		

* All shells were 100% aragonite except the shells of *Crassostrea cf. cucullata* which were 100% calcite.

† Error (±) represents statistical uncertainty derived from counting statistics only.

‡ For the description of the method used for initial detrital ^{230}Th correction, see text.

§ After ref. 8.

|| Slight trace of calcite in an otherwise pure aragonitic shell.

* Extrapolated from Table 2 in ref. 21, which starts at a ^{14}C age of 7,240 yr BP.**Table 2** Comparison of ESR ages with $^{230}\text{Th}/^{234}\text{U}$ and ^{14}C ages for two shells from Tujak

Sample	Species	Archaeological dose (by ESR) (rad)	Tentative annual dose (rad yr ⁻¹)	Tentative ESR-age* (yr BP)	$^{230}\text{Th}/^{234}\text{U}$ age (corrected) (yr BP)	^{14}C age (yr BP)	^{14}C age (calibrated ^{20,21}) (yr BP)
ETIb	<i>Crassostrea cf. cucullata</i>	500	0.103	4,850	2,000	1,700	1,790–1,470
		±150			±500	±105	
ETIc	<i>Asaphis deflorata</i>	900	0.104	8,650	3,400	2,910	3,290–2,840
		±50			±700	±50	

* Annual dose based on CaSO₄: Dy measurements recently carried out by Radtke *et al.*²² on Italian beachrock. Thus the annual dose is only tentative until measurements of U, Th and K on the appropriate matrix material from Tujak have been carried out.

We report here the results of a study designed to allow comparative dating of mollusc shells from beaches which had been raised above sea level by seismic activity or collected for food and thus contained uranium from both marine and terrestrial sources. The key samples were collected from a suite of three Holocene fossil beaches and from a midden in southern Iran, all of which have been reliably dated by ^{14}C (ref. 8). In addition, the analysis was extended to specimens from another marine terrace in the area and one from Qatar. As in the case of the samples used for ^{14}C dating, the material used in the present study consisted of either pure aragonitic or pure calcitic shell carbonate which displayed no evidence of recrystallization, the sole exception being aragonitic shells ETIIb which contained a trace of calcite.

All the uranium-series radiometric data given in Table 1 were obtained by isotope dilution and α -spectrometry techniques described in detail elsewhere⁹. Only the results obtained from homogenized samples composed of several crushed shells are presented. Analyses on single shells in few cases yielded evidence of anomalies due to diagenetic effects such as isotopic exchange with the beach matrix or uranium loss due to leaching after uplift, the coexistence on the seabed of fossil shells subject to isotopically open-system conditions in the marine environment with live organisms which died as a result of uplift and whose shells remained closed systems in their new terrestrial

environment, and reworking of older deposits. The homogenized samples are thus thought to yield more representative results and the following discussion is confined exclusively to these results listed in Table 1.

In this study 0.17 ± 0.01 p.p.m. (all errors quoted here represent 1 s.d. statistical uncertainty) is taken to represent uranium content at zero age. This value, obtained by analysing a modern specimen of *Glycymeris* sp., is in excellent agreement with the mean of 0.18 p.p.m. obtained from the data for 22 living molluscs tabulated by Kaufman *et al.*³. The implication is that the specimens of *Asaphis deflorata*, *Crassostrea cf. cucullata* and *Amiantis hagenowi* display a decrease in uranium content with age, as expected for a system closed to nuclide migration. In contrast, the results obtained for *Scapharca inaequivalvis* indicate postmortem uranium uptake for at least two of the specimens. With the exception of two samples, ETIc and 1A1s, all reported $^{234}\text{U}/^{238}\text{U}$ activity ratios fell within the error bars of the modern specimen activity ratio (1.18 ± 0.07). Thus, in the absence of any correlation between ^{234}U excess and the age of a specimen, the $^{234}\text{U}/^{238}\text{U}$ activity ratios cannot be used to indicate continuous postmortem uranium uptake from non-marine sources^{10,11}.

The presence of ^{232}Th in many marine and terrestrial authigenic carbonates implies a detrital component and hence a certain amount of ^{230}Th not derived from uranium in the

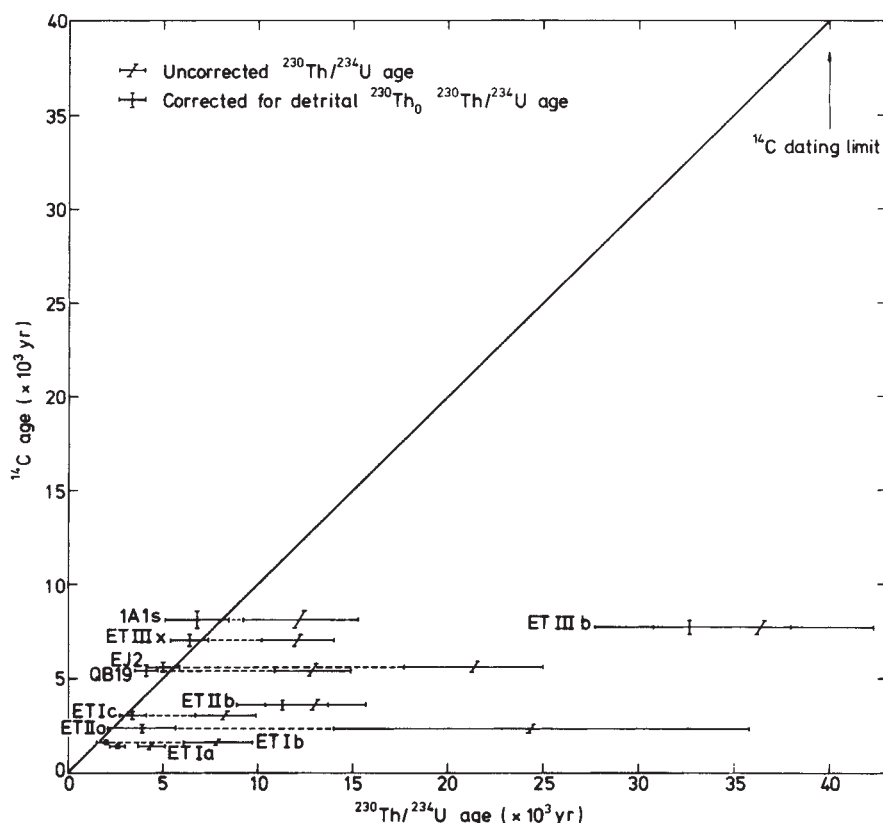


Fig. 1 Plot of $^{230}\text{Th}/^{234}\text{U}$ against ^{14}C ages obtained from mollusc shells from Persian Gulf raised beaches. Inclined ^{14}C bars represent $^{230}\text{Th}/^{234}\text{U}$ uncorrected ages and vertical ^{14}C bars represent $^{230}\text{Th}/^{234}\text{U}$ ages corrected for the presence of detrital ^{230}Th using the method of Kaufman and Broecker¹⁶ (see text for details). Error bars associated with ^{14}C data are limits obtained after ^{14}C dates have been corrected for atmospheric ^{14}C variability (see refs 20, 21). The horizontal bars represent errors (1σ) associated with the $^{230}\text{Th}/^{234}\text{U}$ ages due to counting statistics only.

sample. The accepted criterion is a $^{230}\text{Th}/^{232}\text{Th}$ activity ratio of <20 (refs 1, 12–15). Kaufman and Broecker¹⁶ recognized that some molluscs incorporate ^{232}Th , and by implication ^{230}Th , from their environment¹⁷, and proposed a method for calculating the amount of initially incorporated, unsupported ^{230}Th :

$$(^{230}\text{Th}/^{234}\text{U})_{\text{corrected}} = (^{230}\text{Th}/^{234}\text{U})_{\text{measured}} - (^{230}\text{Th}/^{232}\text{Th})_0 \times (^{232}\text{Th}/^{234}\text{U})_{\text{measured}} e^{-\lambda_{230}t}$$

where $(^{230}\text{Th}/^{232}\text{Th})_0$ is the initial thorium isotopic activity ratio incorporated from the sedimentary matrix, λ_{230} is the decay constant of ^{230}Th , and t is time in years since the death of the mollusc. The corrected age is obtained by setting equal the right-hand side of the above equation and the right-hand side of the $^{230}\text{Th}/^{234}\text{U}$ growth equation (see, for example, ref. 1, equation (3.4)), and solving for t by iteration. The method has been used successfully for dating some shells in Arctic Canada¹¹ as well as *Tridacna*¹⁸. All the samples listed in Table 1 yielded very low $^{230}\text{Th}/^{232}\text{Th}$ activity ratios (2.1 to 15) indicating the presence of detrital thorium. The $^{230}\text{Th}/^{232}\text{Th}$ activity ratio of 1.8 obtained for the modern specimen of *Glycymeris* sp. was used for the initial detrital thorium correction of the original ages (see Table 1). This value is almost identical to the value of 1.7 used by Kaufman and Broecker and obtained from 12 lacustrine samples¹⁶. A plot of both corrected and uncorrected $^{230}\text{Th}/^{234}\text{U}$ ages against ^{14}C ages in Fig. 1 shows excellent agreement between the corrected $^{230}\text{Th}/^{234}\text{U}$ and ^{14}C ages for all but the *S. inaequalis* specimens. Whether this is an effect related to the life habits of the species or an accidental product of the depositional environment is not clear, although it is encouraging to find that such a discrepancy in results could have been foreseen from the evidence for post-depositional uranium uptake given by the high uranium contents of these specimens.

An attempt to apply the ESR dating method¹⁹ to two of the samples from Tujak yielded the results listed in Table 2. Note that the ratio between the two ESR ages is the same as for the ^{14}C and corrected $^{230}\text{Th}/^{234}\text{U}$ ages. The discrepancy in the

absolute ages could stem from an error in postulating the annual dose for the Tujak specimens; the relevant data for that beach are not available. The problem illustrates the need for direct assessment of local environmental effects for all three dating methods.

The excellent agreement between the ^{14}C and $^{230}\text{Th}/^{234}\text{U}$ ages for both calcitic and aragonitic shells after the correction for initial detrital ^{230}Th is encouraging, but it remains to be seen whether the method yields satisfactory results when applied to older specimens not only from the same area but also from climatically different regimes.

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Morphology and structure of biogenic magnetite particles

Tsuyoshi Matsuda, Junji Endo, Nobuyuki Osakabe & Akira Tonomura

Central Research Laboratory, Hitachi Ltd, Kokubunji, Tokyo 185, Japan

Tatsuo Arai

National Institute for Physiological Sciences, Okazaki, Aichi 444, Japan

Blakemore¹ found aquatic bacteria that swim along magnetic lines of force. Such bacteria have small particles of magnetite (magnetosomes) within them^{2,3} of various shapes³⁻⁵. We describe here the morphology and structure of bacterial magnetosomes investigated by high-resolution electron microscopy. From direct observation of various kinds of lattice images, the particles are determined to be single crystals with a hexagonal prism shape truncated by {111} planes. The lattice spacings measured agree with those of magnetite.

We have determined experimentally the crystal habit and magnetic domain structure of bacterial magnetosomes using our field-emission electron microscope⁶. This microscope has the highest lattice resolution⁷ hitherto reported and can observe

magnetic lines of force as phase contour lines of a transmitted electron beam by means of electron holography^{8,9}.

Experiments were carried out using a 125 kV field-emission electron microscope. Magnetotactic bacteria, from the mud in a pond at our laboratory, were attracted into clean water by use of a magnetic field. They were placed on a 100 Å thick carbon film and observed with the electron microscope. The specimens were not stained. An electron micrograph of bacterium (Fig. 1) shows that the fine particles are aligned.

The alignment may have been disarranged during preparation of the specimen but not by the magnetic field of the objective lens in the electron microscope during observation.

Two typical shapes were found, a rectangle and a truncated rectangle, as shown in Fig. 2. Crystal lattices are observed in both images, which indicate a single-crystalline structure of the particles. The fringe spacings of 4.8 Å agreed with those of the (111) and $(\bar{1}\bar{1}1)$ lattice planes of magnetite. Furthermore, it was concluded from the fringes parallel to the particle edges that the particles were covered with facets of crystal lattice planes.

This observation of lattice images leads to the following crystal habit determination for the particles. The two different shapes shown in Fig. 2 are not for different types of particles; the rectangle and truncated rectangle are two views of the same type of particle, in the $[2\bar{1}1]$ and $[0\bar{1}1]$ directions, respectively. Thus, the shape is determined to be a hexagonal prism truncated by {111} planes, as shown in Fig. 2c. The {220} and {311} lattice fringes were also observed in the images of other particles in the expected directions.

This crystal habit was also confirmed using a high-voltage electron microscope, the HU-1250 M (1,000 kV) at the National Institute for Physiological Sciences. A photograph of the view in the $[0\bar{1}1]$ direction is shown in Fig. 3. Lattice planes of (111), $(\bar{1}\bar{1}1)$ and (200) were observed in directions consistent with the predicted structure.

In summary, the bacterial magnetosomes have been determined to be single crystals with outer faces parallel to lattice planes of low indices. They are considered to consist of magnetite, since the spacings and orientations of all the observed fringes agree with those of magnetite². The direction of magnetosome alignment coincides with the primary axis of magnetization in magnetite. Thus the magnetosomes are thought to have single domains and to form a navigating compass⁵.

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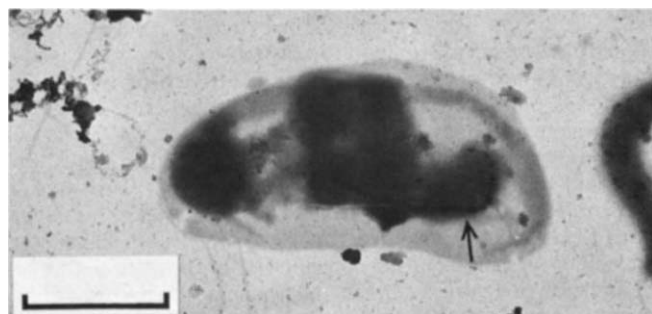


Fig. 1 Electron micrograph of a magnetotactic bacterium, in which fine particles are aligned. Scale bar, 1 µm.

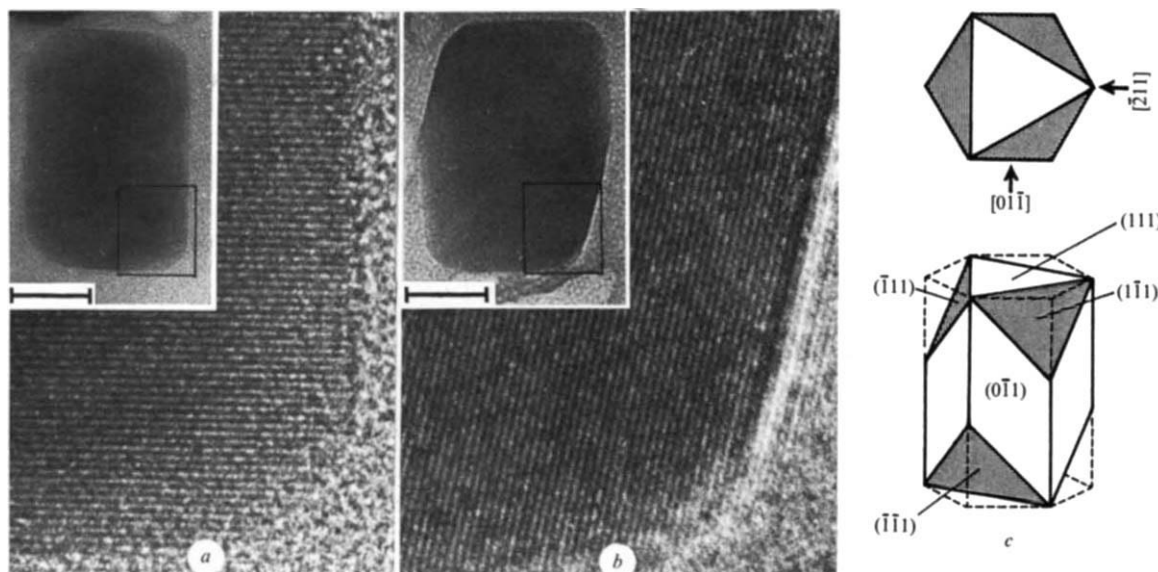


Fig. 2 Electron micrographs of magnetosomes. Typical outer shapes observed are *a*, rectangle and *b*, truncated rectangle. Scale bar, 300 Å. Lattice fringes of 4.8 Å spacing are observed in both images, which correspond to {111} lattice planes of a magnetite crystal. Lattice imaging revealed that these two micrographs were two views in different directions of a single particle illustrated in *c*.

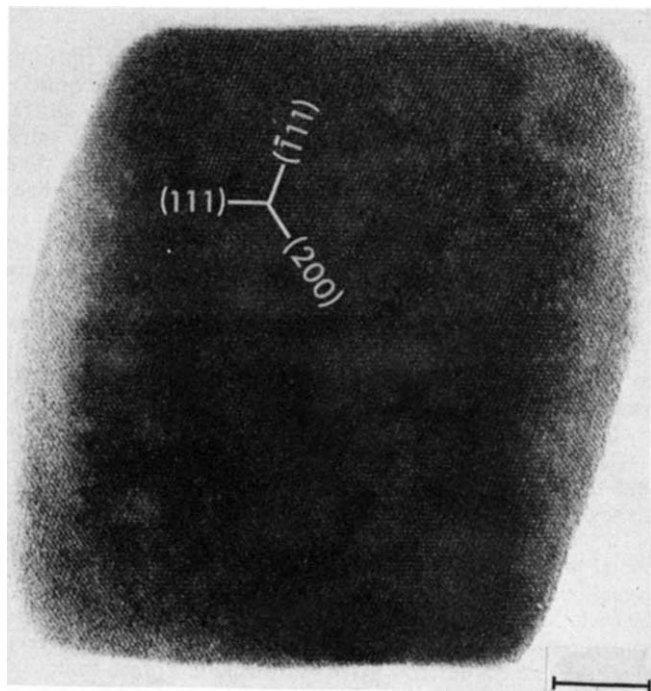


Fig. 3 High-resolution electron micrograph of a particle. Lattice fringes of (111), $(\bar{1}\bar{1}\bar{1})$ and (200) planes can be observed. Their spacings and directions are consistent with the particle model shown in Fig. 2c. Scale bar, 100 Å.

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Photon scattering as a probe of microviscosity and channel size in gels such as sickle haemoglobin

F. Madonia, P. L. San Biagio & M. U. Palma*

Istituto di Fisica dell'Università di Palermo, Palermo, Italy

G. Schiliro', S. Musumeci & G. Russo

Clinica Pediatrica dell'Università di Catania, Catania, Italy

The aggregation of sickle-cell haemoglobin (HbS) is one of the most physiologically important and widely studied macromolecular gelation processes. Both the thermodynamics and kinetics of the process are important in determining the pathological consequences of deoxygenation of the red cells (and both must be understood if a rational strategy is to be developed for pharmacological intervention). We describe here a new and versatile technique for the study of the structure and formation of the HbS aggregates, that should be widely applicable to gel systems generally. We use laser autocorrelation spectroscopy to observe the diffusion of monodisperse polystyrene latex spheres in the interstices of the gel.

* Also at Consiglio Nazionale Ricerche, Istituto Applicazioni Interdisciplinari della Fisica, Palermo, Italy.

Minute concentrations of monodisperse polystyrene latex spheres were used as probes, added to the specimens before independently that they do not interfere with the process of gelation. Then we measured their translational diffusion coefficient as a function of time and temperature, by using photon correlation spectrometry (PCS). Local viscosity and its time and temperature dependence are easily derived from the diffusion coefficients if the latex spheres have a uniform and known hydrodynamic radius, and if the Stokes–Einstein (S–E) relation is valid. As we shall see, this relation is valid in non-gelled systems for spheres of any size within the available range, and in gelled systems for spheres whose diameter is small compared with the transverse sizes of channels.

HbS, obtained from heterozygote donors, was treated by standard techniques, slightly modified as reported previously¹, and separated from other haemoglobins by passage on DEAE cellulose². Acrylamide gel electrophoresis showed a HbS purity better than 99%. Samples were maintained and manipulated at 4 °C. They were deoxygenated and, in oxygen-free conditions, were filtered through a 0.8 µm Millipore membrane directly into the cuvettes to be used for the experiment. These had been prepared by rinsing with Millipore water, drying in a dust- and oxygen-free environment, and depositing in them small quantities of oxygen-free latex sphere suspensions. N-dithionite was then added, its final concentration being kept below 50 mM. The cuvettes were then tightly closed by a Teflon screw cap and O-ring seal. Water from a super-Q Millipore system was used throughout. Water, buffer and dithionite solutions to be used for preparing the HbS solutions for PCS experiments were further filtered through a 0.22 µm Millipore membrane. At the end of the experiments, samples were taken to 0 °C to bring them back to the sol state, and precise spectrophotometric measurements of protein concentration were made. This allowed methaemoglobin (MetHb) formation to be monitored. The invariance of the pH was also checked at the end of the experiments.

The translational diffusion coefficient, D_T , of the probes was measured by PCS using a Malvern K 7023 correlator and a Malvern RR 102 photon correlation spectrometer, together with a Lexel krypton laser tuned at 676.4 nm. The spectrometer cell holder was modified as described elsewhere³ to minimize the path of the laser beam in the specimen and that of the photons in the 90° scattering directions (1 mm each), thus minimizing any difficulties originating from high absorbance at high HbS concentrations⁴. The laser power was maintained at about 10 mW. Samples were irradiated for the short time necessary to accumulate the required photon counts and were then kept in the dark until the next kinetic point was to be taken.

A three- or sixfold increase of laser power and continuous exposure for several hours produced no damage in HbA samples having the same concentration and pH as HbS samples, as shown by the preservation of the protein spectroscopic and functional properties. Spectrophotometric and turbidometric measurements were also performed on HbS samples brought back to the sol state following laser irradiation, PCS experiments and gelation and revealed evidence of no protein damage. In particular, even after a second gelation, MetHbS was consistently found to be well below 4%. Parallel turbidometric measurements were also done, using a Cary 118c spectrophotometer, and determining turbidity from measurements of apparent absorbance at 730 nm, on aliquots of the same HbS samples used for PCS experiments with and without addition of latex spheres, and at the same temperature as for the PCS experiments. Gelation kinetics were identical, and showed no evidence for the occurrence of laser heating, protein damage, or a role of latex spheres in gelation.

Dow Corning polystyrene spheres were used, having nominal diameters of 0.085 µm and 0.498 µm (0.5 µm henceforth). A preliminary check was made that the S–E relation $D_T = kT/6\pi\eta R_H$ (where D_T is the translational diffusion coefficient, k is the Boltzmann constant, T the absolute temperature, and η the viscosity coefficient probed by our spheres of hydro-

dynamic radius R_H) was valid for these spheres in simple aqueous solution. To this purpose we prepared a set of aqueous solutions of ethanol and glycerol in different proportions so as to give macroscopic viscosity values ranging from 0.5 to several hundred centipoise⁵. Latex spheres were added, in concentrations equal to those used in the HbS experiments. PCS measurements showed that the S-E relation was accurately and consistently satisfied when macroscopic values for η and nominal, monodisperse geometric values for R_H were used. This also showed that neither aggregation nor hydration of the latex spheres could be measured by the present method (we shall return to this point below). Note that in buffered solutions polystyrene spheres were observed to aggregate at salt and/or sphere concentrations above certain (low) limiting values; however, these were never approached in our experiments.

Concentrations about 1.5×10^{10} and 8×10^7 particles per cm^3 were used for the 0.085 and 0.5 μm spheres, respectively. These values proved to be an excellent compromise between the requirements of minimum perturbation of the system, minimum probe-probe interaction, and the largest possible signal from the probes. As will be seen in our discussion of the results (particularly those in Fig. 4), the use of the above concentrations and of PCS sample (correlation) times matching the expected probe mobility, allowed correlation functions to be recorded which fitted a single exponential very satisfactorily. Thus, the possibility of obtaining correlation functions related to the translational diffusion of our probes was essentially not affected by the presence of Hb molecules nor by extended gel aggregates or the dynamics of the gel itself⁶, although, of course, they did depend on changes (with time and temperature) in the diffusion coefficient. PCS experimental data provide the intensity correlation function $g^2(t)$. The required information is contained in the field correlation function $g^1(t)$. The relationship between the two, in the (unavoidable) presence of a small number of 'dust' particles and more generally of non-gaussian extra contributions, is⁷

$$g^2(t) - 1 = A^2 [g^1(t)]^2 + C \quad (1)$$

where C is a constant which takes into account all non-gaussian backgrounds. In our case, we used a single exponential function for fitting $g^1(t)$, by imposing

$$A^2 [g^1(t)]^2 + C = A^2 \exp(-2D_T q^2 t) + C \quad (2)$$

and thus obtaining optimized values for amplitude A , diffusion coefficient D_T and background C . (Note that the wavevector q is determined by the experimental arrangement.)

When the S-E relation is valid, D_T values obtained in this way provide numerical values for ηR_H . In this case, if R_H is known, meaningful values for the locally measured viscosity, which we shall henceforth call microviscosity, are immediately obtained. In the following we shall present and discuss experimental results, and provide evidence for the limits of validity of the S-E relation. As we shall see, when the S-E relation loses its validity, data are only qualitatively useful, even if discussed in terms of D_T values, but when the S-E relation is valid, data are best viewed in terms of η values. For this reason we shall present and discuss our experimental results in terms of microviscosity.

Data in Fig. 1 illustrate experimental accuracy and reproducibility for a non-gelling haemoglobin solution in an experiment using 0.5 μm spheres. Here again, the use of this value for R_H gave a microviscosity coincident with that provided by macroscopic measurements⁸. Note that sphere aggregation would here simulate an increase of η value.

Figure 2 compares gelation observed by the present and by standard turbidometric methods, in two experiments run simultaneously on two aliquots of the same preparation. Curve A is a computer-redrawn plot on a logarithmic time scale of the continuously recorded apparent absorbance at 730 nm for a 1 mm thick sample. It shows the usual 'incubation' time. Curve V shows the same incubation time and a subsequent increase

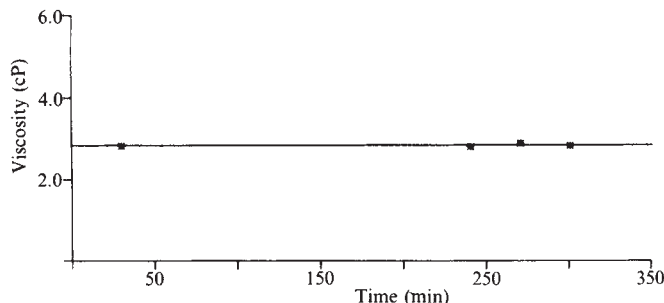


Fig. 1 Time independence of microviscosity (as defined in the text) of a non-gelling HbA₀ aqueous solution. Experimental conditions: concentration 20% w/v; phosphate buffer 0.2 M, at pH 7.0; polystyrene sphere diameter 0.5 μm . At the start of the experiment, the specimen was pulse-heated from 4 °C (preparation and storage temperature) to a controlled 25 ± 0.05 °C.

of apparent microviscosity, rapidly diverging to infinity. This reflects the fall (down to zero within the limits of our observation) in the diffusion coefficient of the 0.5 μm spheres, as they become trapped in the gel structure. As in the case of the characterization of gelation by macroscopic viscosity measurements, the qualitatively vertical stretch of curve V marks the transition to the gel state. Immobilization makes the S-E relation invalid for these probes, but the change in D_T and therefore in the apparent η values during gelation occurs so rapidly that loss of validity of the S-E relation is unimportant for showing qualitatively the occurrence of gelation and measuring the incubation time quantitatively. In this experiment, and for both curves A and V, gelation (entropy-driven and thermoreversible) occurs on pulse heating obtained by quickly transferring the specimens from the storage temperature (4 °C) to the experimental temperature, other conditions remaining unchanged. For both A and V, the transfers were simultaneous and the experimental temperatures identical (25 ± 0.05 °C). Several minutes were allowed after the transfer for thermalization before data acquisition was started (this is a very short time compared with the ~200 min necessary for gelation to occur in the present condition). Some variations of limited amplitude and reproducibility, still well above the noise level, were

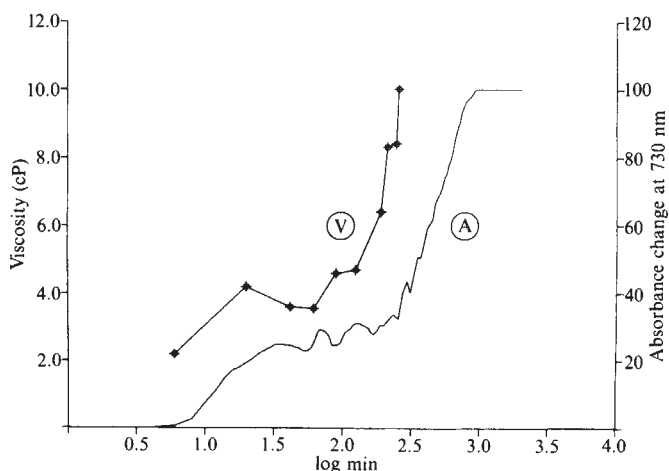


Fig. 2 Time dependence of microviscosity as defined in the text (curve V) and of apparent absorbance at 730 nm due to turbidity (curve A) in deoxyHbS. Curves A and V refer to two simultaneous and independent experiments on aliquots of the same sample treated identically. Experimental conditions and measurements of particle diameter were as for Fig. 1, except that samples were deoxygenated, 50 mM sodium dithionite was added, and cuvettes were tightly closed with a screw cap and O-ring seal. At the start specimens were pulse-heated from 4 °C (preparation and storage temperature) to a controlled 25 ± 0.05 °C.

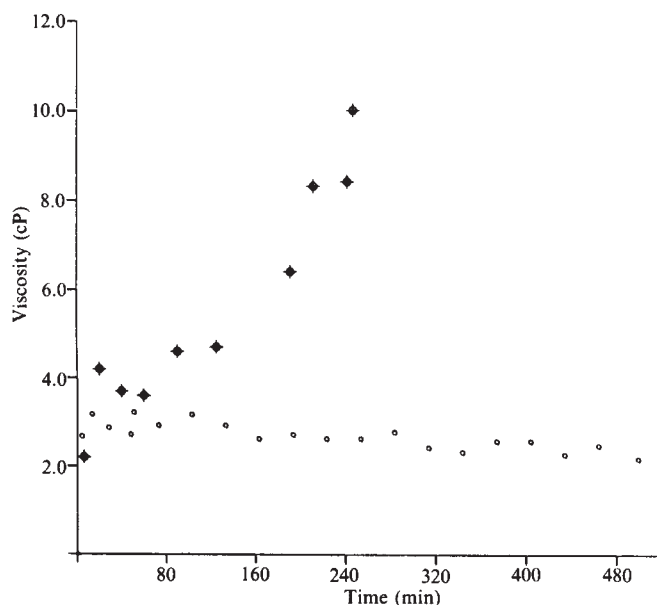


Fig. 3 Comparison between microviscosities of a deoxyHbS aqueous solution as defined in the text and as measured by 0.5 and 0.085 μm spheres. Crossed circles reproduce the same experimental data as in Fig. 2 (0.5 μm spheres). Small circles refer to measurements on 0.085 μm spheres in the identical system, in identical experimental conditions. Macroscopic gelation is observed in this case to start after little more than 2 h. The smaller probes are permanently observed to drift freely (in the present case, up to 8 h after gelation).

repeatedly observed in both curves in Fig. 2 and these are being studied further (manuscript in preparation).

We may ask whether it is reasonable to expect in our experimental conditions that spheres of 0.5 μm diameter would become trapped in the gel structure, as indicated by the observed drop in D_T and the corresponding rise in η . The following very crude computation provides an affirmative answer. In our experimental conditions, the minimum gelling concentration for deoxyHbS is 17/18% w/v, while the actual concentration was 20% w/v. It follows that only about 2/3% of HbS was used to build up the gel structure⁹. Assuming that this consists of HbS fibres having a cross-section of $\sim 200 \text{ \AA}$ ^{10,11} and making the further, extreme, assumption that these fibres are uniformly distributed and aligned, we obtain an average channel size of $\sim 0.2 \mu\text{m}$ diameter. Channels of this size would trap our 0.5 μm probes.

Now, could smaller probes be observed to drift within the (micro)channels? We have already seen that 0.5 μm probes become firmly trapped on gelation. The notion of microviscosity, as we have defined it, loses its meaning for these immobilized probes. In contrast, probes having a diameter of 0.085 μm retain their mobility even after gelation and (hypothetical) readjustments or annealing of the gel structure. The microviscosity value provided in Fig. 3 for these smaller probes coincides (within the experimental errors) with the expected macroscopic value of a Hb solution at 25 $^{\circ}\text{C}$ and just below minimum gelling concentration. This justifies the use of the S-E relation in considering the drift of the smaller probes within microchannels. The viscosity of the non-gelling fraction of the solution trapped in the microchannels can therefore be measured. Note that in these conditions macroscopic gelation starts after slightly more than 2 h, whereas immobilization of the 0.5 μm spheres occurs after about 3 h, and that the free drift of the 0.085 μm spheres is observable permanently (here for 8 h). A slightly negative slope is perhaps perceptible in Fig. 3 for the curve relative to small probes. If real, this could correspond to a hitherto unobserved and much slower gel readjustment and/or continuation of the gelation process. This could lower (slowly but steadily) the concentration of HbS not taking part in the gelation and remaining in the gel channels.

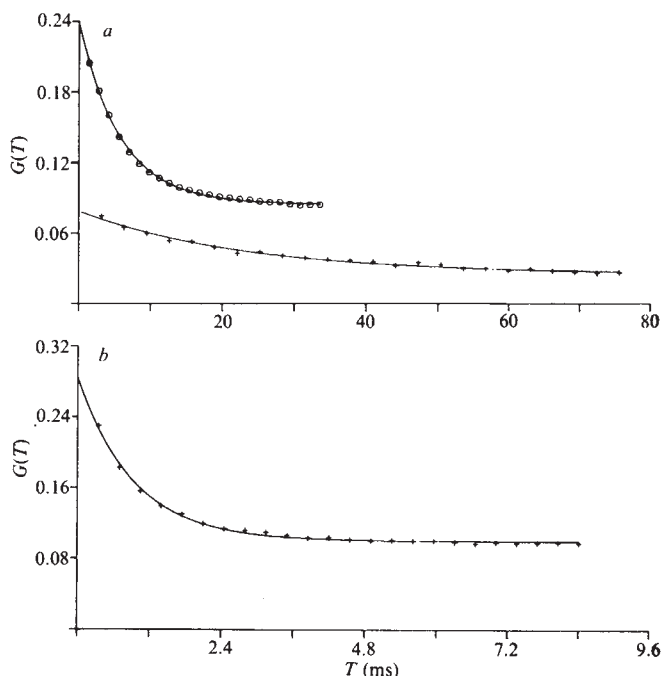


Fig. 4 Fits of experimental data, using equation (2) in the text. *a*, Larger spheres (0.5 μm) in a specimen before the start of gelation (circles) and during gelation (crosses). Note the decreased amplitude of the exponential, showing progressive immobilization. *b*, Smaller spheres (0.085 μm). fits maintain the same quality before and after gelation.

Figure 4 assesses the quality of the fits observed and the consequent reliability of our results. Figure 4*a* concerns the larger particles (0.5 μm) and shows fits of experimental data to a single exponential (equation (2)) before gelation (circles) and while trapping is rapidly occurring (crosses). It also shows the expected amplitude decrease of the exponential as an increasing number of spheres become immobilized. When immobilization is complete, or when gelation in the absence of latex spheres is observed at the same sample (correlation) times, experimental data lie on a flat horizontal line. A typical fit concerning the smaller spheres (0.085 μm) is given in Fig. 4*b*. The amplitude of the exponential shows in this case no measurable dependence on the occurrence of gelation.

A sixfold increase of probe radius (from 0.085 to 0.5 μm) causes complete immobilization in our specific case. This is a much faster transition than that predicted for a random array of interlaced fibres^{6,12,13}. Indeed, when globular HbS tetramers condense into fibres^{10,11} they will be considerably more rigid than, for example, polysaccharide chains¹⁴ and attain a highly anisotropic order¹⁵⁻¹⁹. The very phenomenon of sickling of the otherwise disk-shaped erythrocytes would not occur if gelation were to preserve isotropy. HbS gels can then be viewed in terms of domains of nematic liquid crystals, rather than of ordinary cross-linked gels^{18,19}. This might conceivably give rise to a reduced polydispersity of channel sizes, and consequently to the observed fast 'immobilized-free' transition of probes as a function of their radius.

We now ask whether microviscosity in the gel channels can be measured meaningfully. Coupling by backflow effects is not expected to occur among probes because of their large mutual distance. Standard hydrodynamics, however, predicts wall effects in a channel whose size is not very much larger than a sphere passing through it. An apparent viscosity increase (by a factor of 2 or 3) is expected if the sphere is only three times smaller than the channel²⁰. In fact, Fig. 3 does not show such an increase, although the smaller spheres are only six times smaller than the larger ones, which become immobilized in the gel. Possibly, if not necessarily, one should look for concurrent causes tending to alter viscosity in the microchannels. One interesting possibility is offered by hydrophilic areas on gel

channel walls, which can originate 'long-lived' ($\approx 10^{-9}$ s) connectivity pathways of hydrogen bonds in solvent water. Along these, a non-zero order parameter will exist, to which other water molecules can respond and contribute (refs 21–23 and G. Corongiu, S. L. Fornili and E. Clementi, in preparation). It is possible that this could affect microviscosity²¹. An extreme example of this type of situation is that of a cooperative hysteretic phase transition in a smectic lecithin–water system²⁴. This transition affects the lecithin subsystem and the average hydrogen-bond strength within the water subsystem, the two effects being coupled by crossed feedbacks^{21,24,25}.

In conclusion, the problem of microviscosity in gel channels

deserves further study. This is also suggested by other current problems²⁶. Hopefully, the present approach can be used for more extensive microviscosity, nucleation and gelation studies, and can non-destructively provide information on the texture of a variety of supramolecular structures during their assembly.

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Short-range order of crystallin proteins accounts for eye lens transparency

Mireille Delaye

Laboratoire de Physique des Solides, Bat. 510, Université Paris-Sud, 91405 Orsay, France

Annette Tardieu

Centre de Génétique Moléculaire, CNRS, 91190 Gif-sur-Yvette, France

In its normal state, the eye lens is transparent despite the presence in the cell cytoplasm of high concentrations of proteins, the crystallins, which, *a priori*, could be expected to scatter an important part of the incident light. Early on, an explanation was sought in the spatial correlations between individual scatterers. Trokel¹ first proposed that the "high concentration of proteins in the lens must be accompanied by a degree of local order approaching a paracrystalline state"; Benedek² subsequently suggested that a dense, noncrystalline packing of the proteins would sufficiently reduce the scattered intensity. However, in spite of an improved understanding of the molecular structure of crystallins^{3–6}, their spatial order remained unknown. We present here a small-angle X-ray scattering study of the problem, performed with calf lens cytoplasm both in intact lenses and in cytoplasmic extracts where the crystallin concentration was varied from 3 to 510 mg ml⁻¹. All our experimental data are consistent with short-range spatial order, as in dense liquids or glasses^{7–9}, and this provides a simple explanation for lens transparency^{2,10}. In addition, we detected no conformational change or reorganization of the crystallin proteins throughout the investigated concentration range.

Vertebrate lenses are made of long fibre cells, enclosing as a major cytoplasmic component lens-specific proteins, the crystallins. α -Crystallins (molecular weight $\approx 750,000$ – $1,200,000$) and β -crystallins (β_h , $\approx 180,000$, β_l , $\approx 60,000$) are heterogeneous oligomeric proteins, while γ -crystallins are monomers ($\approx 20,000$)⁵.

Small-angle X-ray scattering experiments can be performed directly on intact lenses. Because cell membranes account for only a few per cent of the dry weight and have a mean electron density lower than that of proteins, the spectra obtained are essentially due to proteins: predominantly crystallins. Because of their high concentration, the crystallins do not behave as independent scatterers. In the X-ray spectra, the scattering of the individual particles is therefore modulated by a structure factor corresponding to the spatial order or, in other terms, to position correlations of the scattering elements⁷. The X-ray pattern of a piece of intact lens is shown in Fig. 1; it displays a broad maximum around $s = 0.0067 \text{ \AA}^{-1}$ ($s = 2 \sin \theta / \lambda$), without any pronounced subsidiary maxima or minima. This maximum is broad enough to rule out the hypothesis of some kind of periodic, long-range ordering of the molecules; in fact, spectra similar to that in Fig. 1 are frequently encountered with dense liquids or glasses^{7–9} characterized by the absence of a regular underlying lattice organization.

To analyse this further, X-ray experiments as a function of concentration are required. As such experiments cannot be carried out on intact lenses, we used cytoplasmic preparations¹¹: undiluted lens cortices (periphery of the lens) are homogenized and ultracentrifuged (40 h, 37 °C, 300,000g) to eliminate membrane fragments; such preparations serve as stock solutions for further dilutions. For identical crystallin concentrations ($C = 430 \text{ mg ml}^{-1}$), the X-ray patterns obtained with the cytoplasmic preparations and intact lenses are very similar (Fig. 1). Neither the conformation of the crystallins (in agreement with ref. 11) nor their spatial order seems to be perturbed by centrifugation. Note, however, that the occurrence in the lens of preferential associations between crystallins and the lens plasma membrane–cytoskeleton complex¹² is not excluded. Figure 1 only means that with or without such associations, the spatial correlations between crystallins remain essentially the same.

Figure 2a shows scattering curves plotted on an absolute scale as a function of crystallin concentration in the range 3–510 mg ml⁻¹. The asymptotic behaviour of the scattering curves, which measures the extent of interface between solvent and scattering particles¹³, does not vary with concentration. This constancy of the asymptotic behaviour strongly argues that the crystallins do not suffer major reorganization or conformational changes as the concentration is varied. Therefore, the scattering elements can be considered to be invariant as a function of concentration.

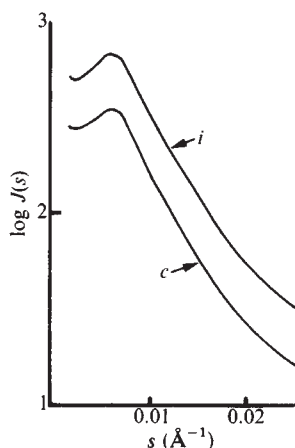


Fig. 1 X-ray patterns of a piece of intact lens (*i*) and a cytoplasmic preparation (*c*) of identical crystallin concentration ($C = 430 \text{ mg ml}^{-1}$) obtained from undiluted cortex. Preliminary experiments on nuclear extracts (that is, from the central part of the lens) gave X-ray patterns similar to *c*. The vertical scales (arbitrary units) are shifted to facilitate comparison. Once subtracted for background and plotted on a linear scale, these patterns look similar to those recorded by Siezen and Berger on α -crystallin gels¹⁵. The scattering experiments were performed with an Elliott rotating anode generator. The data were recorded with a position-sensitive detector²⁰ (the sample-to-detector distance was 50 cm; Δs per channel was $1.8 \times 10^{-4} \text{ Å}^{-1}$, $s = 2 \sin \theta / \lambda$). The experimental procedures were as described elsewhere²¹, experiments being performed at 20 °C. Experiments performed as a function of T for two extreme crystallin concentrations did not show any influence of the temperature between 4 and 37 °C. The weight fractions of the various crystallins in the cytoplasmic preparations were evaluated from HPLC elution patterns (Waters Inc. HPLC, gel filtration column TSK 4000 SW). They are, respectively, $\alpha = 47\%$, $\beta_h = 22\%$, $\beta_l = 22\%$ and $\gamma = 9\%$. Note that ultracentrifugation slightly decreases the β and γ content compared with the initial homogenate¹⁶.

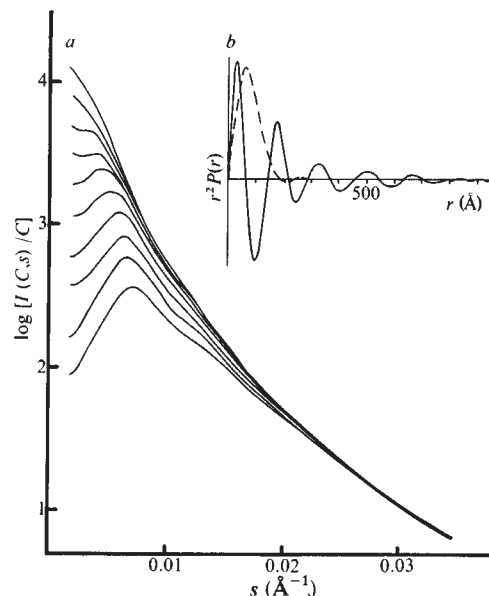


Fig. 2 *a*, X-ray scattering curves, $I(C, s)/C$, recorded with cytoplasmic preparations as a function of concentration. From top to bottom, the protein concentrations are, respectively, 3, 70, 150, 208, 247, 303, 355, 404, 464 and 510 mg ml^{-1} . Additional spectra were recorded in the range 3–150 mg ml^{-1} that are not reported for the sake of clarity. The spectra are corrected for background and collimation distortions and normalized to one electron of solute as in ref. 21. Samples were prepared from the stock solution ($C = 510 \text{ mg ml}^{-1}$) by dilution with a physiological buffer: 0.133 M NaCl, 0.003 M KCl, 0.016 M Na_2HPO_4 , 0.002 M KH_2PO_4 , 1 mM EDTA, 0.2 mM phenylmethylsulphonyl fluoride pH 7.4. *b*, Pair distribution functions $r^2P(r)$ corresponding to the scattering curve extrapolated to infinite dilution (---) and the scattering curve recorded at $C = 404 \text{ mg ml}^{-1}$ (physiological concentration) (—). The vertical scales are arbitrary.

It is consequently possible to analyse the size and shape of the scattering elements from the intensity curve extrapolated to infinite dilution, $\lim_{C \rightarrow 0} I(C, s)/C$, that is, in the absence of spatial correlations^{13,14}. The low angle part of this curve is consistent with the presence of globular particles: Guinier plots give straight lines, whose extrapolation to the origin allows the determination of an average particle molecular weight M ($M = \sum M_i C_i / \sum C_i$, where C_i is the weight concentration of each crystallin species). M is found to be $557,000 \pm 40,000$ (taking the partial specific volume of the crystallins equal to 0.74 g cm^{-3}). The average hydrated volume is found to be $865,000 \pm 60,000 \text{ Å}^3$ (ref. 13). From M and V , the hydration (supposed to be the same for each species) is calculated to be $0.20 \pm 0.03 \text{ g per g}$, a value commonly encountered with globular proteins. The measured radius of gyration, $58 \pm 2 \text{ Å}$, that may be expected to be close to that of the particles with the highest molecular weight, leads to an actual radius of $\sim 75 \text{ Å}$ for a spherical particle. Finally, the maximum diameter of the largest scattering elements, obtained from the point where the pair distribution function $r^2P(r)$ goes to zero¹⁴, is estimated to be $180 \pm 20 \text{ Å}$ (Fig. 2*b*, dotted line).

All these results are consistent with the interpretation that the scattering elements are the weighted mixture of individual α -, β - and γ -crystallins evaluated from HPLC elution patterns (see Fig. 1 legend). In particular, such an analysis leads for the largest scattering elements to an average molecular weight of $1,070,000 \pm 100,000$; this and the other parameters (radius of gyration and maximum diameter) are in good agreement with previous studies of α -crystallins^{3-4,15,16}. These α -crystallins—that is, the largest particles—clearly dominate the scattering: 47% α -crystallins by weight (that is, about 5 α -crystallin molecules together with 95 or so crystallin molecules of lower molecular weight) accounts for 85% of the intensity at the origin.

Returning to Fig. 2*a*, we see that the variations observed in the X-ray patterns, located at small s , are a continuous function of the concentration, devoid of abrupt changes. They thus seem to reflect a progressively closer packing of the lens proteins as the concentration is increased, without any clue of a transition towards some kind of crystalline order. The extent of spatial correlations at each concentration can be estimated from the Fourier transform of the corresponding X-ray spectrum, that is, from the pair distribution function $r^2P(r)$. An example is shown in Fig. 2*b* (solid line) for $C \approx 400 \text{ mg ml}^{-1}$, in the range of physiological concentrations. Pseudo-periodic, featureless oscillations can be seen that may be attributed to correlations between neighbouring crystallins (in fact, α -crystallins as the scattering is dominated by them: note that the period of the oscillations is close to the diameter of an average α -crystallin molecule). Although the functions $r^2P(r)$ are now complicated combinations of terms from the individual scatterers and from their spatial order, the fact that the oscillations are damped as a function of r and disappear for $r \geq 800 \text{ Å}$ indicates that spatial correlations are lost after about four neighbouring layers. Such an extent for spatial correlations of α -crystallins in the eye lens is consistent with the short-range order usually encountered in dense liquids or glasses⁷⁻⁹ and has also been observed with concentrated solutions of other biological macromolecules¹⁷. A further argument in favour of limited spatial correlations is provided by the comparison of the evolution of the intensity at the origin as a function of concentration, $I(C, 0)$, with that calculated for various models. These intensities at the origin were obtained from extrapolation of the X-ray curves, neglecting any possible 'macroscopic' density fluctuations within the sample (that is, fluctuations having Fourier components of the order of $1,000 \text{ Å}$ or more, see Fig. 3 legend). In Fig. 3, the $I(C, 0)$ values are compared with the simplest model of short-range order: a fluid of non-attracting 'hard spheres'^{18,19}. The

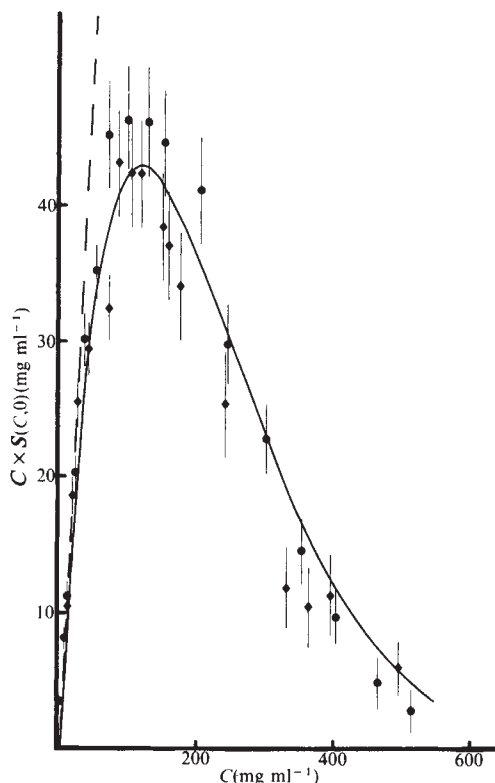


Fig. 3 Evolution of the intensity at the origin (X-ray forward scattering) as a function of concentration. The normalized intensity scattered at s by a solution of concentration C may be written⁷

$$I(C, s)/C = \{\lim_{C \rightarrow 0} I(C, s)/C\} \times S(C, s) \quad (1)$$

where the term in brackets is the limit, at vanishing concentration of the normalized intensity and $S(C, s)$ is a structure factor describing the spatial correlations. We have plotted here, as a function of C , the values at the origin of $I(C, s)/\{\lim_{C \rightarrow 0} I(C, s)/C\}$, that is $C \times S(C, 0)$. Note that all the values are given on an absolute scale. ●, Extrapolated X-ray data. For low concentrations ($C < 50 \text{ mg ml}^{-1}$), the values of $I(C, 0)$ were obtained from extrapolations to the origin of Guinier plots⁷. For higher concentrations, we extrapolated $S(C, s)$ instead of $I(C, s)$ (A.T. and M.D. in preparation). Indeed, if we neglect possible macroscopic density fluctuations (that is, if the density averaged over $1,000 \text{ \AA}$ or so is constant through the sample), $S(C, s)$ is bound to display near the origin a smooth and monotonic variation, which is not the case for $I(C, s)$. ♦, Light scattering data ($\lambda = 5,145 \text{ \AA}$) taken from ref. 10. The average molecular weight determined by these experiments is $535,000 \pm 80,000$, in excellent agreement with the X-ray data. —, Values of $C \times S(C, 0)$ calculated for a fluid of hard spheres. $S(C, 0)$ is proportional to the isothermal compressibility^{7,22}. Using Carnahan and Starling's formula^{18,19} for the osmotic pressure, one obtains for the hard sphere model¹⁰

$$S(C, 0) = (1 - \phi)^4 / (1 + 4\phi + 4\phi^2 - \phi^3 + \phi^4) \quad (2)$$

In equation (2), ϕ is the excluded volume fraction of the solute. The best agreement between experimental and calculated data was found for $\phi = 1.1 C/1,000$. This corresponds to an average excluded volume per particle 1.15 times larger than the average hydrated volume, which seems reasonable for a large oligomeric protein like α -crystallin. The small discrepancies between experimental and calculated data may probably be attributed to the oversimplification of the model that postulates spherical particles, homogeneous in size. ---, Values expected in the absence of spatial correlations ($S(C, 0) = 1$).

striking agreement between experimental and calculated data once again argues for a short-range spatial order analogous to that of dense liquids. Figure 3 also compares the X-ray data with intensities of visible light ($\lambda = 5,145 \text{ \AA}$) scattered by the same series of cytoplasmic preparations¹⁰. The coincidence between X-ray and light scattering data argues for the absence of macroscopic density fluctuations and moreover indicates that light scattering data—most relevant to a discussion of lens

transparency—are entirely accounted for by the liquid-like short-range order analysed with X-ray experiments.

Quantitative conclusions about lens transparency can now be drawn from Fig. 3. If crystallins were independent scatterers, the scattered intensity would be proportional to protein concentration (dotted line in Fig. 3). As calculated by Trokel¹, 70% of the visible light would then be scattered, giving the lens a turbid aspect. Figure 3 demonstrates that the spatial correlations between crystallins at physiological concentrations ($C \approx 400 \text{ mg ml}^{-1}$) reduce the intensity $I(C, 0)$ to only 2.5% of that of independent scatterers (that is, to 2% of the incident light). As already suggested by Benedek², it is a short-range, liquid-like or glass-like order of the crystallins in the lens that accounts for eye lens transparency.

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Activation of rhodopsin phosphorylation is triggered by the lumirhodopsin–metarhodopsin I transition

R. Paulsen & J. Bontrop

Abteilung Allgemeine Zoologie (Biologie I), Universität Ulm, Postfach 4066, D-7900 Ulm, FRG

The absorption of light by the chromophore of rhodopsin initiates a series of interconversions between spectrally distinct intermediates^{1–3}. The possibility has been raised that one of these transitions is accompanied by a change in the state of rhodopsin, and that it is this change which instigates visual excitation via a cascade of enzyme catalysed reactions. It has been suggested that the initial step of this cascade, which leads to the activation of cyclic GMP phosphodiesterase (PDE), involves the interaction of a GTP-binding regulatory (G) protein with rhodopsin^{4–6}. The ability of rhodopsin to activate PDE may be inhibited by the phosphorylation of sites exposed on the opsin surface as a result of light-induced conformational changes^{7–9}. To obtain more information about the relationship between the postchemical and biochemical reactions of rhodopsin we have investigated which transition leads to the activation of rhodopsin as a substrate for rhodopsin kinase, and report here that it is the transition from lumirhodopsin to metarhodopsin I.

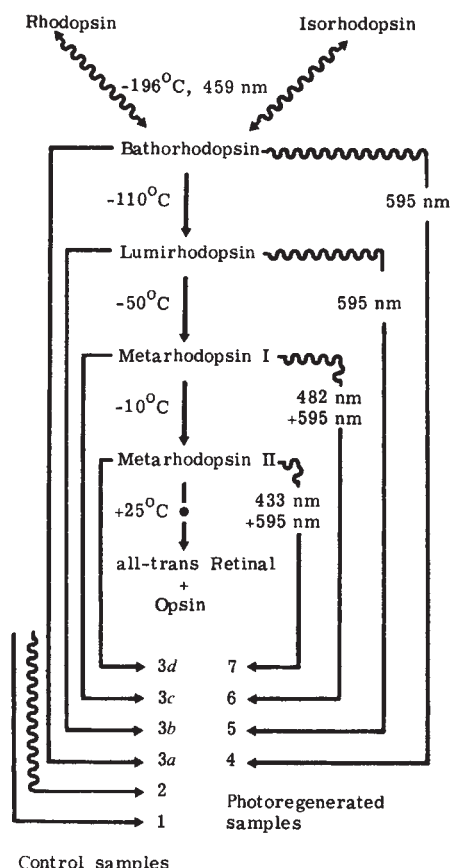


Fig. 1 Scheme for formation of rhodopsin intermediates and photoregeneration of rhodopsin in isolated ROS at low temperatures. Irradiation steps are indicated by wavy lines, and dark reactions by straight lines. For low temperature experiments, ROS suspended in an isotonic salt solution (see Fig. 3) were frozen at -196°C in a small plastic vessel, in which they formed a thin layer adhering to the wall of the vessel. If the temperature had to be raised above -196°C , the sample was placed in an isolated cuvette and exposed to a flow of nitrogen, the temperature of which could be adjusted automatically within the range from -140°C to -10°C in less than 5 min to within 5°C of the desired temperature. Irradiation was performed with a 500 W light source with heat absorbing and narrow band interference filters (Schott) in the light path. Regeneration from metarhodopsin I was carried out at pH 8.3 to reduce the probability of metarhodopsin II being formed; sample 4 and control sample 3a were readjusted from pH 8.3 to pH 6.8 after warming to room temperature. ROS of photoregenerated samples and control samples were kept at -196°C before assaying phosphate incorporation from $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ at 25°C .

Previous work indicated that the phosphorylatable conformation of rhodopsin is adopted before the formation of the long-lived intermediate metarhodopsin III^{10,11}. Furthermore, it has been shown that this active conformation is not inactivated by the regeneration of rhodopsin from opsin and 11-*cis* retinal^{11,12}, nor by photoregeneration from thermally stable cephalopod metarhodopsin¹³. Accordingly, the experiments reported here were designed assuming that the step at which the rhodopsin is activated can be revealed by assigning rhodopsin photoregeneration from short-lived intermediates. Intermediate formation and photoregeneration were carried out at low temperatures, enabling the transitions to be blocked at particular intermediates. The programme for formation of intermediates and photoregeneration of rhodopsin was based on transition temperatures and maximum absorbance of intermediates reported by Kawamura *et al.*¹⁴ (for details see Fig. 1). The experiments were performed with isolated, buffer-washed rod outer segments (ROS), obtained by standard procedures from dark adapted frog (*Rana esculenta*) retinas.

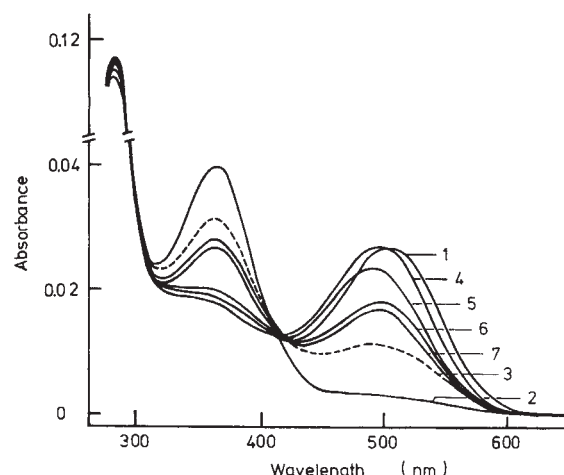


Fig. 2 Bleaching and photoregeneration in isolated ROS at low temperatures. Curve 1, absorbance of the dark control (sample 1). Curve 2, absorbance of the light control (sample 2), 90% rhodopsin bleached at 25°C . Curve 3, photostationary state with bathorhodopsin present after irradiation with 459 nm at -196°C . The curve is also representative of control samples in which intermediates were not photoreconverted (samples 3b-d). Curve 4, absorbance after regeneration from bathorhodopsin. Curve 5, absorbance after regeneration from lumirhodopsin. Curve 6, absorbance after regeneration from metarhodopsin I. Curve 7, absorbance after regeneration from metarhodopsin II. Absorbance spectra were recorded after solubilizing ROS aliquots from samples 1-7 (see Fig. 1) at 4°C in 0.04 M CTAB, 0.15 M hydroxylamine, pH 6.8.

First, we established a photostationary state, consisting of rhodopsin, isorhodopsin and bathorhodopsin³ (formerly pre-lumirhodopsin²), by irradiation of ROS with blue light (459 nm) at the temperature of liquid nitrogen (-196°C). The intermediates that follow bathorhodopsin (see Fig. 1) were obtained by warming this preparation from -196°C to approximately 20°C above the transition temperature of each particular intermediate. Second, at each step of the sequence, the intermediate accumulated in a 40-min dark period was photoregenerated to rhodopsin/isorhodopsin. This photoregeneration was performed at -196°C so that the photoregenerating light produced only bathorhodopsin from rhodopsin/isorhodopsin still present in the sample. Bathorhodopsin formed by the photoregenerating light was also reconverted to rhodopsin/isorhodopsin (Fig. 1, samples 6, 7). Third, photoregenerated ROS samples (samples 4-7) and control samples containing ROS not exposed to a photoregenerating light (Fig. 1, samples 1-3d) were warmed from -196°C to 25°C during a 3-min dark period and, after addition of 5 mM $[\gamma\text{-}^{32}\text{P}]\text{ATP}$, assayed for phosphate incorporation in the dark^{11,12}. To estimate the amount of rhodopsin bleached or rhodopsin/isorhodopsin regenerated, a portion of each ROS sample was solubilized at 4°C in 0.04 M cetyltrimethylammonium bromide (CTAB) containing 0.15 M hydroxylamine.

As photoregeneration at -196°C produced a mixture of rhodopsin and isorhodopsin the relevant spectra are shifted from 504 nm to shorter wavelengths (Fig. 2, compare curve 1 with curves 4-7). In ROS exposed to a photoregenerating light there was a significant increase in the concentrations of rhodopsin/isorhodopsin (curves 4-7) compared with the control samples (represented by curve 3).

Light-induced incorporation of phosphate into ROS is shown in Fig. 3. Having confirmed that for each ROS preparation the maximum phosphate incorporation was linearly related to the number of rhodopsin molecules bleached by an orange light at 25°C (Fig. 3 inset), we interpret the effects of rhodopsin/isorhodopsin regeneration on phosphorylation as follows. Photoregeneration from bathorhodopsin and lumirhodopsin produced a high concentration of rhodopsin/isorhodopsin (Fig. 3,

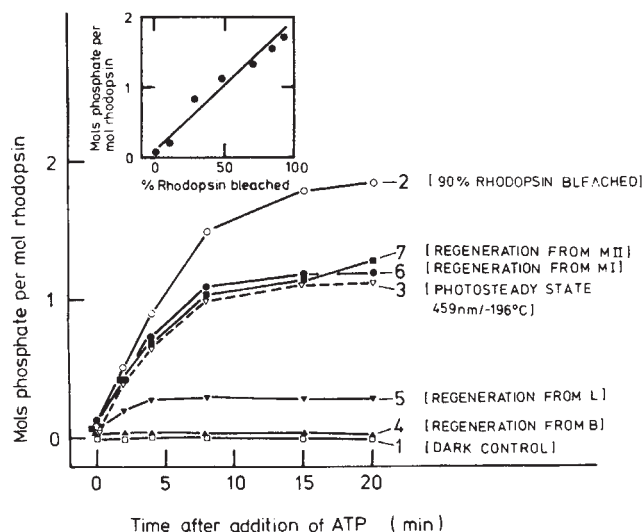


Fig. 3 Light-dependent phosphate incorporation into isolated ROS. Curve 1, dark control (sample 1). Curve 2, light control (sample 2). ROS of light and dark controls were kept at -196°C before assaying phosphate incorporation at 25°C . Curve 3, photosteady state with bathorhodopsin present (459 nm , -196°C); average values of samples 3a–d with s.d. less than $\pm 10\%$. Curve 4, after regeneration from bathorhodopsin (B). Curve 5, after regeneration from lumirhodopsin (L). Curve 6, after regeneration from metarhodopsin I (MI). Curve 7, after regeneration from metarhodopsin II (MII). The inset shows the relation between the amount of rhodopsin bleached by an orange light at 25°C and phosphate incorporation measured 16 min after addition of ATP. The values for phosphate incorporation after 16 min (inset) and 20 min (time courses) are the mean of three measurements and had a s.d. of less than $\pm 10\%$. Phosphate incorporation was measured at 25°C in an isotonic solution containing 115 mM NaCl , 2 mM MgCl_2 and 10 mM HEPES , $\text{pH } 6.8$, after addition of $5\text{ mM } [\gamma\text{-}^{32}\text{P}]\text{ATP}$. Details of the irradiation programme are given in Fig. 1.

curves 4, 5), and suppressed phosphorylation to the level measured in dark-adapted ROS (Fig. 3, compare curves 4, 5 with curve 1). Thus, formation of these intermediates was not accompanied by a conformational change converting rhodopsin into a substrate for rhodopsin kinase. In contrast, photoregeneration from metarhodopsin I and metarhodopsin II did not affect phosphorylation (Fig. 3, compare curves 3, 6, 7). Therefore, the conformation of rhodopsin necessary for an interaction with rhodopsin kinase must have been induced by molecular events occurring during the lumirhodopsin–metarhodopsin I transition.

Even though rhodopsin phosphorylation is triggered by the lumirhodopsin–metarhodopsin I transition, the exposure of phosphate binding sites may occur at a later stage of the intermediate sequence, for example during the metarhodopsin I–metarhodopsin II transition. However, most of the available data suggest that the stoichiometry and time course of the subsequent phosphorylation are independent of the classical rhodopsin intermediate and regeneration sequence¹². Furthermore, neither dephosphorylation nor the inactivation of the phosphorylatable conformation of opsin seem to be affected by later changes in the interactions between retinal and opsin^{8,12,15}. Thus, the transition initiating the formation of a phosphorylatable opsin conformation is the only step in the activation of rhodopsin that can be correlated with a spectral intermediate change.

The binding of negatively charged phosphate groups may affect the interaction of opsin with other proteins, for example the G-protein complex. Accordingly, phosphorylation of rhodopsin may be involved in the rapid, ATP-mediated reversal of PDE activation, as has recently been suggested by Liebman and Pugh⁷. If rhodopsin phosphorylation terminates an amplification step in the transduction process, photoregeneration from intermediates before the metarhodopsin I stage

should interrupt the reaction sequence, leading to a desensitization of the visual cell, whereas photoregeneration from the metarhodopsin I stage should not affect the sensitivity control mechanism. This is, in principle, what has been shown by double flash experiments for the events leading to an elevation of visual thresholds of rods in the frog retina¹⁶ and in the human eye¹⁷. Thus, as previously suggested^{12,15,18}, phosphorylation of rhodopsin may be part of a mechanism which modulates visual sensitivity. 'Rushton's paradox'—the failure of the Dowling–Rushton equation to account for human rod dark adaptation after a photoregenerating flash^{16,19,20}—is then explained by our finding that photoregeneration from intermediates after lumirhodopsin–metarhodopsin I transition restores the spectral identity of rhodopsin without reversing the conformational changes that trigger the phosphorylation of rhodopsin.

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What does the eye see best?

Andrew B. Watson

Department of Psychology, Stanford University, Stanford, California 94305 and NASA-Ames Research Center, Moffett Field, California 94035, USA

H. B. Barlow & John G. Robson

Physiological Laboratory, Cambridge University, Cambridge CB2 3EG, UK

Our eyes see so much in such varied conditions that one might consider the question posed in the title to be meaningless, but we show here that, within the range that we have been able to test, there is a particular spatiotemporal pattern of light that is detected better than any other. At least two plausible theories of visual detection predict that a stimulus will be seen best (will have greatest quantum efficiency) when it matches the weighting function of the most efficient detector. We have measured quantum efficiency for detecting a wide variety of spatiotemporal patterns using foveal vision in bright light. The best stimulus found so far is a small, briefly exposed circular patch of sinusoidal grating having a spatial frequency of $\sim 7\text{ c deg}^{-1}$, drifting at $\sim 4\text{ Hz}$. We propose that this is the weighting function of the most efficient human contrast detector. We believe this answer to the question is unexpected and may have fundamental implications with regard to the mechanisms of visual perception.

A detector is a theoretical entity which maps each presentation of a visual signal into an internal representation on which the observer's decision is based. As a visual signal is distributed

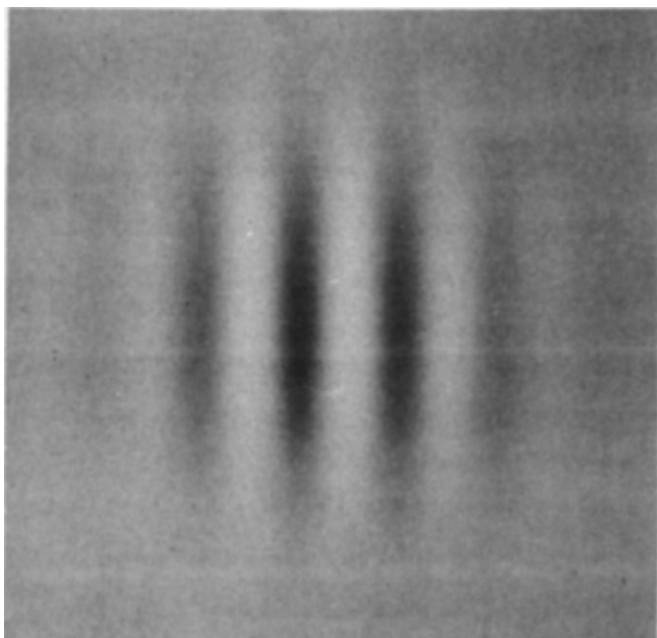


Fig. 1 An example of a grating patch. When viewed from a distance such that one cycle of the sinusoid subtends 1 deg of visual angle, its parameters are: $f_x = 1 \text{ c deg}^{-1}$, $s_x = 1.5 \text{ deg}$, and $s_y = 1.5 \text{ deg}$. For this static photograph, $f_t = 0 \text{ Hz}$ and $s_t = \infty$.

over space and time, any detector must collect together and appropriately combine information at different points in the image at different times. One important class of detectors performs this combination linearly: at each point in space and time, the signal is weighted by some coefficient and these values are added together. The weighting function specifying these coefficients completely characterizes the detector. If this weighting function is $w(x, y, t)$ and the signal is $I(x, y, t)$, then the response R of the detector, before the decision stage, is given by

$$R = \iiint w(x, y, t) I(x, y, t) dx dy dt \quad (1)$$

In the case of a visual neurone, the spatial weighting function corresponds to what is ordinarily called the receptive field sensitivity profile, and there is evidence for linear summation of small signals for many retinal ganglion cells¹⁻⁴ and neurones of the primary visual cortex^{5,6}.

The performance of a visual detector is ultimately limited by noise, part of which is inherent in the quantum fluctuations in the stimulus, and part of which may be due to the physical components of the detector. If this noise is independent from point to point and moment to moment, then there is a well known method for optimally combining information from the image. This optimum is achieved by weighting each point in proportion to its individual signal-to-noise ratio. Hence if the signal is $I(x, y, t)$, then the optimal linear weighting function is $kI(x, y, t)$ where k is any constant. This optimal detector is said to be 'matched' to the signal.

The best any visual detector can ever do is to meet the limit set by quantum fluctuations. This performance therefore represents an ideal, and performance of any detector relative to this ideal may be expressed as an efficiency. Specifically, suppose that the signal is some intensity perturbation, $I_s(x, y, t)$ that is added to an otherwise uniform steady background, $I_N(x, y, t) = I_N$. On signal trials, the intensity in the image is

$$\begin{aligned} I_{S+N}(x, y, t) &= I_S(x, y, t) + I_N \\ &= I_N[1 + c(x, y, t)] \end{aligned} \quad (2)$$

where $c(x, y, t)$ is the contrast at each point. Responses to

signal-plus-noise and noise-alone trials are

$$\begin{aligned} R_{S+N} &= \iiint w(x, y, t) I_{S+N}(x, y, t) dx dy dt \\ &= I_N \iiint w(x, y, t) dx dy dt \\ &\quad + I_N \iiint w(x, y, t) c(x, y, t) dx dy dt \end{aligned} \quad (3)$$

and

$$R_N = I_N \iiint w(x, y, t) dx dy dt \quad (4)$$

The difference between these responses (the portion due to the signal alone) is

$$\begin{aligned} R_S &= R_{S+N} - R_N \\ &= I_N \iiint w(x, y, t) c(x, y, t) dx dy dt \end{aligned} \quad (5)$$

Where c is small, as it is in these experiments, the variance of these responses will be approximately equal and given by the variance at each point (which is simply I_N when intensity is expressed in quanta $\text{deg}^{-2} \text{s}^{-1}$) multiplied by the square of the weight at that point and summed:

$$V = I_N \iiint w^2(x, y, t) dx dy dt \quad (6)$$

However it is measured, performance will be monotonic with the ratio $R_S/\sqrt{V}$, usually called the signal-to-noise ratio or d' . As noted above, for the ideal detector, the weighting function is matched to the signal, in which case

$$\begin{aligned} d'_{\text{ideal}} &= \left[I_N \iiint c^2(x, y, t) dx dy dt \right]^{1/2} \\ &= \sqrt{I_N E} \end{aligned} \quad (7)$$

E is the contrast energy of the signal: the integral of the square of the contrast over all the dimensions in which it varies. Quantum efficiency (F) is the square of the ratio of empirical and ideal d' values for a given contrast energy, or equivalently the ratio of ideal and empirical contrast energies, $E_{\text{ideal}}/E_{\text{actual}}$, at some fixed value of d' (refs 7-9),

$$\begin{aligned} F &= (d'_{\text{actual}}/d'_{\text{ideal}})^2 \quad \text{for constant } E \\ F &= E_{\text{ideal}}/E_{\text{actual}} \quad \text{for constant } d' \end{aligned} \quad (8)$$

We use the latter relation as in our experiments we varied contrast to yield a fixed value of d' . This expression also makes it clear that for any visual signal on a fixed background, efficiency is inversely proportional to threshold contrast energy. Hence that signal is seen best (seen with greatest efficiency) for which threshold contrast energy is least.

Quantum efficiency for a particular signal will be less than one if the detector must contend with noise in addition to quantum fluctuations. However, provided that the noise is effectively uniform over the spatial and spectral extent of a signal, the optimal detector will still be one whose weighting function is matched to the signal.

Our principal motive in asking what the eye sees best is that the preceding observation may be inverted: if only a single detector exists, then the form of the signal that is seen best identifies the weighting function of the detector¹⁰. If on the other hand there are several detectors with different weighting functions, then the best signal would identify the weighting function of the most efficient detector—that least impeded by noise. Therefore the search for the best signal is a search for the weighting function of the most efficient visual detector.

In our experiments we used a two-interval forced-choice procedure. One interval contained I_N , the other I_{S+N} . To perform optimally, the observer would choose that interval in which the detector gave the larger response. An alternative decision rule that has interested us is to select that interval in which the response exceeds some threshold, and if this occurs

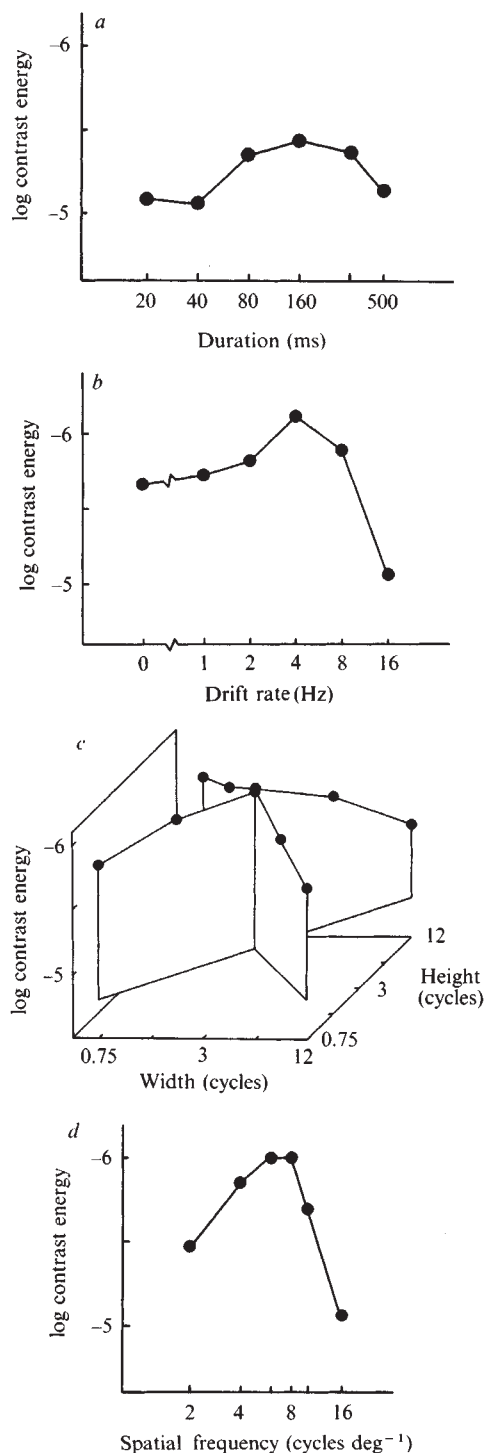


Fig. 2 Contrast energy thresholds for grating patches as a function of duration (a), drift rate (b), height and width (c), and spatial frequency (d). Height, width and duration of a patch are defined as twice the relevant half-widths [see equation (8)]. Standard deviations are ~ 0.1 log unit. Data are for H.B.B.

in neither interval, to guess. Threshold is set so that it is never exceeded in both intervals. Fortunately, it is also true for this detector that contrast energy is least when the signal is matched to the detector weighting function¹¹. Thus here too, when only one detector exists, that signal will be seen best that is matched to the detector weighting function. When several detectors exist, possibly with different thresholds, the best stimulus will identify the weighting function of the detector with lowest threshold. As these thresholds are presumably set by the relevant noise, it is clear that these two formulations are quite similar.

In our search for the best stimulus we have confined ourselves to two classes of patterns, the first being square increments with two adjustable parameters: width and duration. The second class consists of patches of drifting sinusoidal gratings with gaussian envelopes in horizontal, vertical and temporal dimensions. A drifting patch with unit contrast may be written

$$c(x, y, t) = \sin[2\pi(f_x x - f_t t)] \exp[-(x/s_x)^2 - (y/s_y)^2 - (t/s_t)^2] \quad (9)$$

The patch has five parameters: the spatial frequency f_x , the drift frequency f_t , and the horizontal, vertical and temporal gaussian half-widths s_x , s_y and s_t . The width, height or duration of a patch is defined as twice the relevant half-width. An example of a grating patch is shown in Fig. 1.

This second class of pattern is more general than it may at first seem. With appropriate choice of parameters, it includes circular spots of various sizes and thin lines of various lengths. Patches were used in part for their generality, and in part because they include waveforms which resemble the weighting functions of simple cortical cells^{5,6}.

All signals were superimposed on a background of 340 cd m^{-2} , and were computer-generated on a large ($20 \times 30 \text{ cm}$) cathode ray tube with a ^{31}P phosphor. Viewing was binocular with best optical correction and natural pupils from a distance of 228 cm. Observers fixated the centre of the square increment or of the stationary spatial gaussian window. We measured thresholds for two observers (H.B.B. and A.B.W.) with a two-interval, forced-choice staircase procedure.

For square increments, we measured thresholds for squares ranging in width from 0.075 to 1.2 deg, and varying in duration from 10 to 400 ms. The best square we have found has a 0.3 deg side, and a duration of 50 ms. For observer H.B.B., it has a threshold contrast energy of $-5.6 \text{ log deg}^2 \text{ s}$, which corresponds to a threshold contrast of 2.36%. Squares as small as 0.075 deg on a side were detected almost as well.

To find the best grating patch, we must hunt through a five-dimensional space. We have necessarily limited ourselves to a number of slices through this space, and can show here only a few of the many we actually examined. Figure 2a shows contrast energy thresholds for a 10 c deg^{-1} patch, 0.3 deg in height and width, drifting at 4 Hz. We have also measured thresholds for flickering, rather than drifting patches. For the stimuli in Fig. 2a, drifting and flickering patches have almost the same threshold contrast energy. A duration of $\sim 160 \text{ ms}$ is best, although the maximum is not very sharp. Figure 2b shows the dependence on temporal frequency of threshold contrast energy for a 6 c deg^{-1} patch, 0.5 deg in height and width, with an optimal duration of 160 ms. The best frequency is $\sim 4 \text{ Hz}$. Figure 2c illustrates the effect of height and width on the threshold contrast energy of a 6 c deg^{-1} patch with optimal drift rate and duration of 4 Hz and 160 ms. Minimum threshold contrast energy occurs at a width and height of ~ 3 cycles. From comparable results at other spatial frequencies, we find the optimum to be more nearly a fixed number of cycles than a fixed size in degrees. Thus, in Fig. 2d we show contrast energy thresholds for patches of various spatial frequencies in which height and width are fixed at the optimal value of 3 cycles. Duration and drift rate are again set to optimal values of 160 ms and 4 Hz. The optimum spatial frequency is found to lie between 6 and 8 c deg^{-1} . Figure 1, when viewed from such a distance that one cycle of the sinusoid subtends about $1/7$ th of a degree, is a static picture of what the eye sees best.

This is the most efficiently detected stimulus we have discovered. If visual thresholds are set by linear detectors, this approximates the weighting function of the most efficient among them. It is likely that the threshold for a given stimulus is not set by a single detector at a single point in time, but rather by probability summation (or some other variety of nonlinear combination) over various detectors and over time^{12,13}. A more precise estimate of the detector weighting function would take this into account.

For observer H.B.B., the best stimulus has a threshold contrast energy of $-6.03 \text{ log deg}^2 \text{ s}$, corresponding to a threshold

contrast of 1.44% at 6 c deg^{-1} . This is about one-third the threshold contrast energy of the best square. We have not investigated all possible patterns, so a still more visible stimulus may exist at the luminance we have used. We invite those with candidate stimuli to test them against our best.

Note that at the moderately high luminance we have used (340 cd m^{-2}), the actual quantum efficiency is only ~ 0.0005 ($d' = 1.273$ and for both pupils together, $I_N = 4.2 \times 10^9$ quanta $\text{deg}^{-2}\text{ s}^{-1}$). Much higher levels of quantum efficiency have been obtained at lower photopic luminances, and at low scotopic luminances the value approaches the fraction of quanta effectively absorbed¹⁴. As the foveal cones must surely absorb a much higher fraction of the available quanta, there are clearly limits to sensitivity beyond the quantum fluctuations. Recent measurements of thresholds for grating patches embedded in computer-generated noise¹⁵ suggest near-perfect statistical efficiencies for signals like our best. Although noise added to the stimulus does not necessarily mimic internal noise, this observation reinforces our suspicion that the visual brain contains detectors with weighting functions of this form, and strengthens our belief that intrinsic noise limits detection on high luminance backgrounds.

The particular form of our best stimulus is of interest for several reasons. First, the detector spatial weighting function deduced here resembles the receptive field sensitivity profiles of many cortical neurones. The spatial frequency bandwidth of our best detector is about one half-octave, although stimuli with one-octave bandwidth are seen almost as well. Movshon *et al.*⁵ and De Valois *et al.*⁶ found that about 15% of simple cells in area 17 of cat and monkey have bandwidths of one octave or less. Second, we note that the detectors discovered here resemble those derived from other recent experiments on both detection^{16,17} and discrimination^{18,19} and may account for a broad range of previous psychophysical results.

Note also that quantum efficiency changes rather slowly with change of some stimulus parameters near the optimum. This not only makes the search for the best stimulus more difficult, but also suggests that the brain may contain many such detectors, with weighting functions located at different points and suited to stimuli of different parameters. In this regard we observe that the waveform of the best stimulus is one of a set of elementary basis functions devised by Gabor²⁰ and subsequently shown by Helstrom²¹ to provide a complete and exact representation of an arbitrary waveform. Therefore a set of such detectors, when distributed appropriately over space and spatial frequency, are able to encode an arbitrary visual image, as suggested in several recent models^{17,22,23}. Thus patterns like that in Fig. 1 may be among the elementary features of visual perception.

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Pollinator behaviour and natural selection for flower colour in *Delphinium nelsonii*

Nickolas M. Waser & Mary V. Price

Department of Biology, University of California, Riverside, California 92521, USA, and Rocky Mountain Biological Laboratory, Crested Butte, Colorado 81224, USA

Evolutionary biologists continue to disagree about the relative importance of natural selection, drift and phylogenetic constraint in determining characteristics of an organism¹. Because of the difficulty of identifying examples of selection in nature there are few rigorous field studies of selection^{2–6}. We have been studying selection on flower colour in the small perennial larkspur *Delphinium nelsonii*, a native to mountains of the western USA. Previously we showed that white-flowered forms, which are very rare in natural populations, produce fewer seeds than their common blue-flowered conspecifics, and that this selective disadvantage results from partial discrimination against white flowers by bumblebee and hummingbird pollinators⁷. Here we present evidence that discrimination occurs because white flowers have inferior 'nectar guides' and therefore require longer handling times than blue flowers. Pollinators may thus experience lower net rates of energy intake on white flowers, a sufficient reason for undervisitation by optimally-foraging animals.

We studied foraging by introducing bumblebees or hummingbirds into a small flight cage and presenting them with mixtures of potted plants. In these conditions both pollinator types undervisited white-flowered plants, even when they predominate

Table 1 Differences in pollinator behaviour between blue and white flowers

Pollinator and flower type	Handling time ($\bar{X} \pm 1\text{ s.e.m. (N)}$)	
	Between flowers (s)	Within flowers (s)
<i>a, Untreated flowers</i>		
1. <i>B. appositus</i>		
Blue flowers	1.86 ± 0.14 (54)	3.36 ± 0.19 (106)
White flowers	2.05 ± 0.17 (36)	3.97 ± 0.38 (62)
2. <i>B. nevadensis</i>		
Blue flowers	2.04 ± 0.21 (3)	2.73 ± 0.31 (7)
White flowers	2.96 ± 1.47 (3)	3.73 ± 0.91 (5)
3. <i>S. rufus</i>		
Blue flowers	0.38 ± 0.03 (25)	0.72 ± 0.09 (51)
White flowers	0.44 ± 0.02 (58)	1.00 ± 0.11 (89)
4. <i>S. platycercus</i>		
Blue flowers	0.38 ± 0.02 (59)	0.65 ± 0.05 (96)
White flowers	0.41 ± 0.02 (57)	0.93 ± 0.10 (82)
<i>b, Painted flowers—S. platycercus</i>		
1. Before painting		
Blue flowers	0.52 ± 0.08 (16)	0.94 ± 0.16 (46)
White flowers	0.61 ± 0.07 (25)	1.82 ± 0.23 (35)
2. After painting		
Blue flowers	0.36 ± 0.05 (14)	0.62 ± 0.05 (56)
White flowers	0.33 ± 0.04 (30)	0.59 ± 0.05 (69)

Times taken to fly between successive flowers on a single plant (between flowers) and to extract nectar from single flowers (within flowers) in the flight cage experiments discussed in Fig. 1. Basic experiments used queens of two bumblebee species, *Bombus appositus* and *B. nevadensis* (body mass about 0.65 g and 0.75 g, respectively), and males of two hummingbird species, *Selasphorus rufus* (rufous) and *S. platycercus* (broad-tailed) (body mass about 3.5 g each). The flower painting experiment involved a single broad-tailed male. Only after painting are handling times for white flowers shorter than for blue. The probability of obtaining this pattern by chance alone is small ($P < 0.001$, sign test).

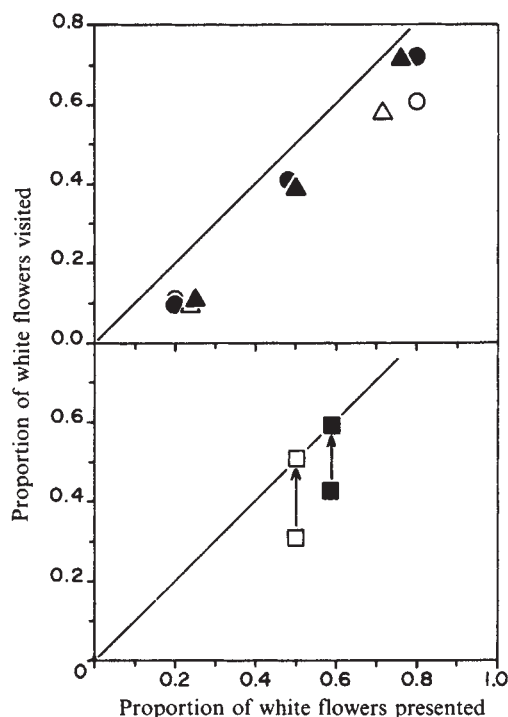


Fig. 1 Pollinator discrimination in flight cage experiments. *a*, $\circ$, Mean proportions of plants visited by bees relative to proportions presented, Δ , the same for bees and flowers (from four experimental replicates of 2:8 and 8:2 mixtures yielding totals of 365 visits to plants and 823 visits to flowers). $\bullet$, $\blacktriangle$, Analogous results for birds and plants or flowers, respectively (from three replicates of 2:8 and 8:2 mixtures and one of 5:5 mixtures yielding totals of 779 visits to plants and 1,376 visits to flowers.) *b*, $\square$, Connected by an arrow, proportions of plants visited by a single hummingbird before and after all flowers were painted to match a normal colour pattern. $\blacksquare$, Proportions of flowers visited (from a single replicate yielding 165 visits to plants and 286 visits to flowers). The straight line in both graphs is the line of no preference. In most experiments a single hummingbird or two or three bumblebee queens were presented with either a 2:8 or 8:2 mixture of blue- and white-flowered potted plants matched for inflorescence height and flower number; in one case a hummingbird and a 5:5 mixture were used. Proportions of blue and white flowers deviated slightly from proportions of plants in each experiment, since matching for flower number was not perfect.

in a mixture; the extent of discrimination is comparable to that found in our previous outdoor experiments⁷ and is remarkably similar for bees and birds (Fig. 1, top).

Various optimality models⁸⁻¹¹ predict partial discrimination if pollinators are under selective pressure to maximize net rates of energy intake while foraging, and if they have lower intake rates on white than on blue inflorescences. Differences in intake rates would occur if white flowers contain less concentrated or smaller amounts of nectar, or if pollinators take longer to extract the same nectar from white than blue flowers. Our earlier studies⁷ showed that nectar concentrations and secretion rates are not consistently different between flower colour morphs, but suggested that handling and extraction times might indeed be different.

To explore the possibility that handling times differ between colour morphs we filmed foraging bouts of bees and birds in the flight cage, and counted individual film frames to measure accurately the times required to visit and extract nectar from single flowers ('within-flower' time) and to fly between successive flowers on single inflorescences ('between-flower' time). Within-flower times are between 1.1 and 1.5 times longer for white- compared with blue-flowered plants, while between-flower times are a factor 1.2 and 1.4 times longer; these ratios hold for all pollinators even though bees and birds differ considerably from each other in body mass (Table 1a). Although



Fig. 2 Flowers of *D. nelsonii* photographed next to a greyness reference scale after the method of Kevan¹⁶. Illumination was diffuse sunlight. Top photograph was taken through a quartz lens without filter; centre photograph through an 18A filter with peak transmittance at 360 nm (near UV); bottom photograph through a 25 filter with peak transmittance at 600 nm (red). At the left top and bottom of each photograph are pistillate phase and staminate phase white flowers, respectively, from the same plant; to the right is an equivalent arrangement of blue flowers. The petals on the face of the blue flowers, which form a distinct reflective target in the bottom photograph, also reflect strongly down to about 500 nm. In contrast, these petals absorb UV light which surrounding petaloid sepals reflect, as seen for flowers of both colours in the centre photograph.

absolute handling times vary between experimental replicates (perhaps as a function of pollinator size, nectar standing crops, and other variables) the qualitative differences associated with flower colour are consistent. The time required to process one white flower and fly to the next is thus 1.2–1.7 times longer than for blue flowers.

What could explain handling time differences? The spurred petals that contain nectar are no longer or more constricted on white than on blue flowers, nor are white flowers more widely spaced along inflorescences (Table 2); thus the mechanical difficulty of nectar extraction should not vary with flower colour.

Table 2 Comparison of morphological features of blue and white flowers

Morphological feature	Blue flowers (mm)	White flowers (mm)
1. Length of nectar spur	1.94 ± 0.03 (20)	1.98 ± 0.04 (20)
2. Maximum width of spur	0.27 ± 0.01 (20)	0.28 ± 0.01 (20)
3. Distance of lowest flower from central inflorescence stalk	2.88 ± 0.26 (13)	2.94 ± 0.22 (16)
4. Straight-line distance between lowest and next-lowest flowers	4.81 ± 0.45 (14)	4.38 ± 0.43 (16)
5. Height difference between lowest and next-lowest flowers	3.01 ± 0.26 (14)	2.41 ± 0.32 (16)

Values are expressed as in Table 1. There are no significant differences related to flower colour for any feature ($t < 1.4$, $P > 0.1$ in all cases). Comparisons of features other than those shown here give similar results.

One possibility is that pollinators cannot handle white flowers efficiently and discriminate against them because they lack experience with these normally rare morphs. If this were the case, however, we should see consistent changes in handling times and preferences as pollinators become more experienced with white in our flight cage experiments. On comparing behaviours at the beginning and end of single experimental runs (which lasted > 8 h), we find no consistent changes either in degree of discrimination against, or handling times on, white flowers. Given the ability for rapid learning by bees and hummingbirds^{12,13}, this suggests that lack of experience with a morph is an unlikely explanation for our results. A more subtle possibility is that white flowers lack the nectar guide of contrasting colour that is provided by two white petals which frame the entrance to concealed nectaries on the face of normal blue flowers. Such nectar guides are found in congeners of *D. nelsonii*^{14,15}, and their absence might make the nectaries of white flowers difficult to locate and hence could slow down pollinators in landing on flowers and inserting mouthparts. This is likely to influence within- as well as between-flower components of handling time. Photographs through a series of monochromatic filters covering wavelengths from near UV to red¹⁶ show that petals on the face of blue flowers provide a contrasting target in all wavelengths but that white flowers provide such a target only in near UV light, which is absorbed by the petals but reflected by the surrounding petaloid sepals (Fig. 2).

Since both bumblebees and hummingbirds are sensitive to near UV light^{13,17,18}, this reflectance pattern should provide white flowers with a nectar guide, but one that covers a smaller range of wavelengths than that of blue flowers. To see whether this difference influences behaviour, we exposed a hummingbird in our flight cage to an equal mixture of blue- and white-flowered plants. As usual, this bird showed partial discrimination against white flowers and took longer to handle them than blue flowers (Table 1b, Fig. 1, bottom). We then removed plants and painted both white and blue flowers with acrylic paint to match the normal pattern of dark blue-purple petaloid sepals surrounding white, unpainted petals. The paint differs from the natural petaloid sepals in that it absorbs UV, but this effect was identical for all flowers. When painted flowers were returned to the flight cage there were no preference and handling time differences between flowers that were originally of different colours (Table 1b, Fig. 1, bottom).

These results suggest that natural selection against albinos results neither from their rarity nor their overall colour, but instead from the reduced colour contrast of flower parts. White-flowered plants seem to provide a subtly inferior advertisement to the location of their concealed nectar. This lowers their effective quality as a food source for pollinators, thus leading to undervisitation and underpollination. If further experiments confirm this interpretation, we will have provided a detailed adaptive explanation for a widespread floral trait: the contrasting colour patterns that Sprengel first interpreted as guides to concealed rewards^{19,20}.

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Malaria parasites— discovery of the early liver form

J. F. G. M. Meis, J. P. Verhave, P. H. K. Jap*,
R. E. Sinden† & J. H. E. T. Meuwissen

Department of Medical Parasitology, Geert Grooteplein Zuid 24, and *Department of Cytology and Histology, Geert Grooteplein Noord 21, University of Nijmegen, 6500 HB Nijmegen, The Netherlands

† Department of Pure and Applied Biology, Imperial College, Prince Consort Road, London SW7 2AZ, UK

Infections of mammalian malaria parasites start when sporozoites from an infected anopheline mosquito are injected into the bloodstream of the host. The sporozoites enter the hepatocytes and become transformed into exoerythrocytic schizonts. Since the discovery of the primate parasite *Plasmodium cynomolgi* in monkey hepatocytes¹ and the rodent parasite *Plasmodium berghei* in hamster hepatocytes², the ultrastructure of these stages has been extensively studied both in primate^{3,4} and rodent plasmodia^{5–10}. These observations relate only to the development of the exoerythrocytic schizont 25 h⁸ after sporozoite injection until the final maturation (of *P. berghei*) 50 h post-inoculation. Recently, we have studied the route of entry of sporozoites across the cellular lining of liver sinusoids and invasion of the liver parenchymal cells^{11–13} by using transmission electron microscopy. The results of these studies in combination with other physiological experiments^{14–17} strongly suggested that the sporozoite was initially harboured by the Kupffer cell, from which the parasite escaped into the neighbouring hepatocyte. The migration of sporozoites from liver sinusoids to hepatocytes can be achieved within a few minutes¹⁸. We present here the first ultrastructural observations on the natural transformation of intrahepatocytic sporozoites into exoerythrocytic forms *in vivo*, using the rodent malaria parasite *P. berghei* in a laboratory host, the Brown Norway rat. These observations complete the search for the final link in the life cycle of malaria parasites.

Sporozoites of *P. berghei* ANKA were collected from *Anopheles stephensi* as described previously¹⁹ and suspended in normal rat serum^{20,21}. Four-week-old female Brown Norway rats were then inoculated with 25 million sporozoites directly into the portal vein. The flow of the portal blood was restricted to the two right liver lobes by ligation of the blood vessels

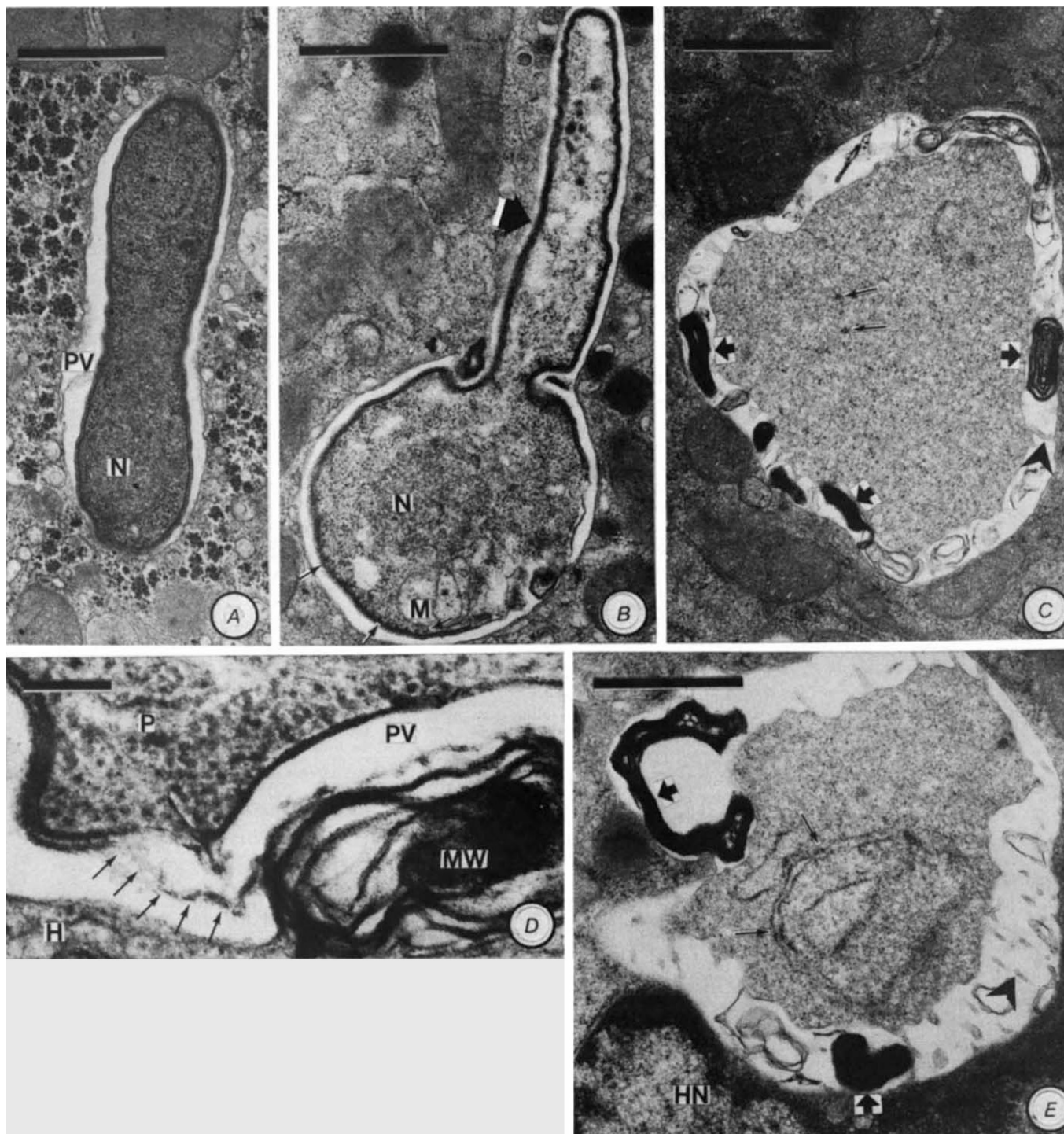


Fig. 1 Electron micrographs of different developmental stages of the exoerythrocytic mammalian malaria parasite *P. berghei* in rat hepatocytes. **A**, Parasite within hepatic cytoplasm 1 h after inoculation. The sporozoite is clearly localized in a membrane-bound parasitophorous vacuole (PV) surrounded by host cell glycogen and mitochondria. Scale bar, 1 μ m. **B**, The early trophozoite 4 h after inoculation of sporozoites. The parasite is still localized in a parasitophorous vacuole, and the perinuclear area, including the mitochondrion (M), has enlarged, whereas the other 'pinched-off' region of the parasite has become much smaller (thick arrow) and less well organized. N, nucleus. The microtubules (thin arrows) emphasize the sporozoite-like nature of this form. The main difference compared with the 1-h form is the partial rupture of the inner pellicle membranes and the appearance of membrane-whorls in the parasitophorous vacuole. Scale bar, 1 μ m. **C**, This parasite, found 10 h after inoculation of sporozoites, differed completely in size, shape and morphology from the 1- and 4-h forms. It lies in a parasitophorous vacuole filled with membrane-whorls (thick arrows) and projecting thin parasite pili (arrowhead). The latter were attached to the host membrane enclosing the parasitophorous vacuole. In the typical electron-lucent parasite cytoplasm, only polysomes (thin arrows) were visible. Scale bar, 1 μ m. **D**, A high magnification of the parasite (P)-host (H) interface 4 h after inoculation. In the parasitophorous vacuole a membrane-whorl (MW) seems to be contiguous (arrows) with both the parasite plasmalemma and the host membrane and may therefore be derived from both structures. Scale bar, 0.1 μ m. **E**, Section of a 10-h parasite lying in a clear parasitophorous vacuole, showing membrane-whorls (thick arrows) and parasite pili (arrowhead). The host nucleus (HN) was indented due to the rapidly enlarging parasite. Apart from some endoplasmic reticulum (thin arrows) and a few polysomes, no parasitic organelles were visible. Scale bar, 1 μ m.

leading to the median, left lateral and caudal lobes⁸. In view of the speed with which sporozoites enter the hepatocytes¹⁸ and their subsequent rapid development and multiplication

(within 50 h), the transformation into exoerythrocytic forms was expected to occur within 10 h of inoculation. Rats were therefore killed 1, 4 and 10 h after inoculation of sporozoites.

The livers were perfused with 1.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.3, 350 mosM) for 5 min at a rate of 2 ml min⁻¹. The right liver lobes were postfixed in osmium tetroxide, dehydrated and embedded in resin (Epon). Areas around the afferent portal tracts were selected for ultra-thin sectioning because these seem to be the 'preferred' sites for exoerythrocytic schizont development¹³.

Sporozoites or sporozoite-derived trophozoites were found to lie in parasitophorous vacuoles of hepatocytes at 1, 4 and 10 h after inoculation (Fig. 1). The identity of the early forms was confirmed by the presence of the typical, trilaminar pellicle and the subpellicular microtubules (Fig. 1A, B), and by the fact that similar structures have never been detected by us in the livers of uninfected animals²². The 1-h parasite still had all the morphological properties of a sporozoite—elongate shape, subpellicular microtubules, rhoptry-microneme complex and pellicle—whereas the 4-h form showed obvious changes. The part of the sporozoite which contained the nucleus and mitochondrion (Fig. 1B) was enlarged and rounded (diameter ~1.8 µm). At some places the outer pellicle membrane became detached and gave rise to membrane-whorls in the parasitophorous vacuole (Fig. 1D). The remaining part of the sporozoite became smaller (diameter <0.5 µm) and appeared to be 'pinched-off' or folded (Fig. 1B). Possibly the latter part was less well organized and might not be involved in the differentiation process. The apical complex was not observed in this part nor was it found to be retracted in the bulbous area. Subpellicular microtubules were still visible in the bulbous part, indicating that these rigid structures are not broken down until later in the process of dedifferentiation. The loss of apical organelles, pellicular rigidity, and the rounding up of the parasite resemble closely the events described in the transition of the highly differentiated merozoite into a structurally simple intraerythrocytic trophozoite²³, and of the ookinete into the oocyst²⁴.

The 10-h form (Fig. 1C, E) has a morphology clearly intermediate between the sporozoite and the 25-h exoerythrocytic form⁸. It bears little resemblance to the sporozoite (Fig. 1A) and is larger than the parasite recorded in Fig. 1B (2.5 µm compared with 1.8 µm). The inner pellicle membranes and microtubules of the sporozoite have been lost in the areas viewed, leaving a single plasma membrane. The originally dense cytoplasm had assumed an electron-lucent appearance (typical of the maturing liver schizont⁸ and the early erythrocytic trophozoite²³) with scattered ribosomes and few polysomes. The endoplasmic reticulum (Fig. 1E) is expanded over that seen in the sporozoite but has not yet proliferated to the extent seen in the 25-h schizont⁸. Unfortunately, the rarity of material has prevented a more detailed description of this stage. The parasitophorous vacuole became enlarged, as is also the case in parasitized erythrocytes, immediately after merozoite invasion. Pili of parasitic origin (Fig. 1C, E) were seen projecting into the vacuole where they made contact with the host membrane. These prominent structures have not been observed in the other schizogonic/sporogonic stages of the life cycle; their temporary appearance may be related to the transfer of nutrients across the large vacuolar host-parasite barrier. Membrane-whorls, as seen during penetration of the sporozoite, at 1 and 4 h of intrahepatocytic life, remained visible in the vacuole at 10 h. Similar lamellar bodies were described in vacuoles of erythrocyte-penetrating merozoites of *Plasmodium knowlesi*²⁵. These were thought to originate from secreted rhoptry content.

The present findings emphasize the repetitive and conservative nature of plasmodial development in its different environments and host cells²⁶. We note that the dedifferentiating sporozoite has certain similarities with the hypnozoite of *P. cynomolgi* as observed by light microscopy^{27,28}, suggesting that it is at this stage that relapsing plasmodial species enter their latent phase.

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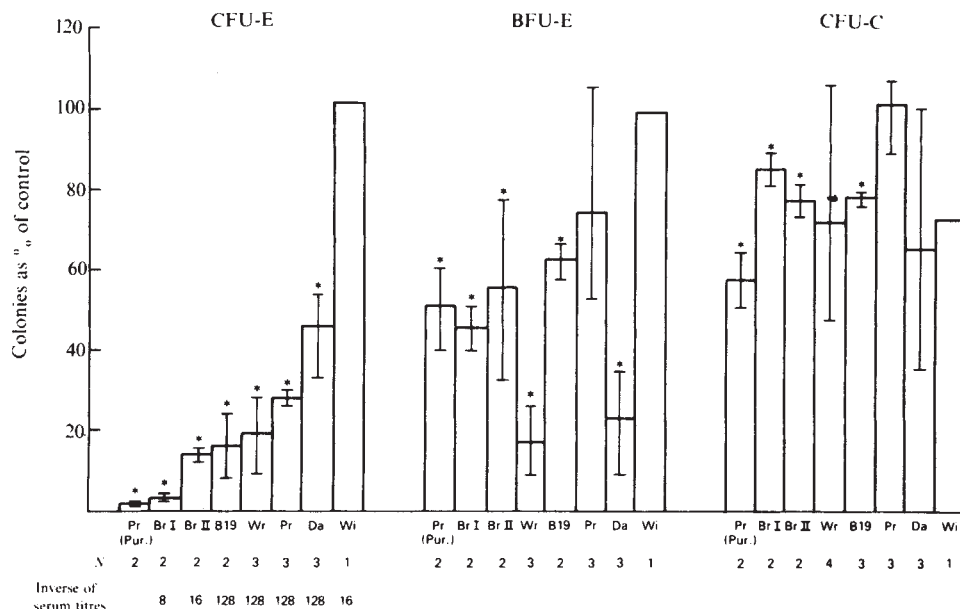
A human parvovirus-like virus inhibits haematopoietic colony formation *in vitro*

Philip P. Mortimer*, R. Keith Humphries†, Jeffrey G. Moore†, Robert H. Purcell* & Neal S. Young†

* Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases and † Clinical Hematology Branch, National Heart, Lung, and Blood Institute, Bethesda, Maryland 20205, USA

Viruses have been shown to cause bone marrow aplasia in animals^{1,2} and have been implicated in bone marrow failure in man³⁻⁶; however, until recently, a specific link between human viral infection and bone marrow failure has not been proven. In 1975 Cossart and colleagues found a serum parvovirus-like virus (SPLV, sometimes referred to as B19) in human serum⁷. Antibody to this virus is present in the sera of 30-45% of healthy adults (Y. E. Cossart, P. P. Mortimer, unpublished observations). However, evidence for a direct link came from work by Pattison *et al.* who found five children with transient aplastic crisis of sickle cell disease and evidence of active infection with SPLV⁸. This association was later confirmed in a large series of children with sickle cell disease and aplastic crisis in Jamaica⁹. We have studied the effects of virus-containing material on haematopoiesis, using *in vitro* colony-forming assays to look for direct evidence for a role of SPLV in bone marrow aplasia. We show here that SPLV-containing sera inhibit erythropoiesis in culture. Moreover, in a child with hereditary spherocytosis who developed transient aplastic crisis, a strong inhibitory effect of the patient's serum on erythropoiesis correlated with the presence of virus.

Fig. 1 Effect on subsequent haematopoietic colony formation of incubating normal human bone marrow cells with sera containing SPLV. Individual serum samples are encoded by abbreviations (Pr, Br I, Br II, Wr, B19, Pr, Da, Wi) and include a purified preparation of one serum (Pr). Sera positive for SPLV or negative control sera were diluted to 10^{-2} in Iscove's modification of Dulbecco's modified Eagle's medium (IMDM) and $150 \mu\text{l}$ added to 3×10^5 cells in a total volume of $165 \mu\text{l}$. Cells and SPLV-containing sera were incubated for 4 h at 4°C and examination showed that there was no aggregation or loss of cells during incubation. The mixture was directly added to 3 ml of culture media consisting of 0.8% methylcellulose, 30% fetal calf serum (heat inactivated for 30 min at 56°C), 1% bovine serum albumin, 10% phytohaemagglutinin-stimulated leukocyte conditioned medium, 2.5 U ml^{-1} erythropoietin (Connaught) in IMDM. 1-ml aliquots containing 10^5 cells were plated in duplicate in 35-mm standard dishes (Lux) and incubated at 37°C in 5% CO_2 in humidified air. After 8 and 14 days, coded incubation dishes were scored twice for CFU-E, BFU-E and CFU-C derived colony formation. Mean (bars) and range (vertical lines) of colony counts from one to four experiments for each serum specimen are shown expressed as a % of the mean counts obtained from two to four SPLV-negative control sera tested in parallel. *. Values obtained in these experiments are significantly different ($P < 0.05$) from control values by Student's *t*-test. *N* represents number of experiments in which each sera was tested. SPLV antigen titres were assayed by counter-current immunoelectrophoresis.



Seven sera were available that had previously been found to contain SPLV by counter-current immunoelectrophoresis (CIE) and electron microscopy. Five, from voluntary blood donors, were detected in the course of screening for hepatitis B virus surface antigen⁷ and two were obtained from patients with brief febrile illnesses¹⁰. To determine their effects on human haematopoietic cells, 10^{-2} dilutions of the sera were incubated with normal bone marrow cells for 4 h at 4°C ; the bone marrow cells were then cultured in methylcellulose to assay erythroid and myeloid colony formation (Fig. 1). Control sera, obtained from healthy donors, were similarly tested. Three haematopoietic progenitor types were assayed by their distinctive colony characteristics¹¹ as follows: (1) colonies derived from the later erythroid progenitor, the erythroid colony forming unit (CFU-E), were counted on day 8 and characterized by 16–64 haemoglobin-containing cells in a tight cluster; (2) the more primitive erythroid progenitor, the burst-forming unit (BFU-E), was determined on day 14 by counting colonies consisting of three or more clusters of hundreds of haemoglobin-containing cells; (3) granulocyte-macrophage progenitors (CFU-C) were determined by counts of colonies with no haemoglobin-containing cells on day 14. In each experiment, colony counts were expressed as a percentage of the mean counts derived from two to four control sera tested in parallel. For each serum sample tested, the mean and range obtained as well as the number of experiments performed and the serum SPLV antigen titres by CIE are shown. Six out of seven SPLV-containing sera showed more than 50% mean inhibition of CFU-E derived colony formation. Three out of seven sera resulted in similar levels of inhibition of erythroid colony formation from the more primitive progenitor, the BFU-E, but none inhibited formation of myeloid colonies to this degree. It was not possible to detect viral antigen in cell lysates or tissue culture media taken from suspension cultures of bone marrow cells following inoculation with SPLV. This was perhaps due to the difficulty of maintaining late erythroid progenitors in liquid tissue culture¹². A virus-rich fraction of serum was prepared by Dr B. J. Cohen of the PHLS Virus Reference Laboratory, London, by elution from Sephacryl S-300 and pelleting through a 20% sucrose cushion. This resulted in at least an eightfold increase in antigen titre compared with the original serum

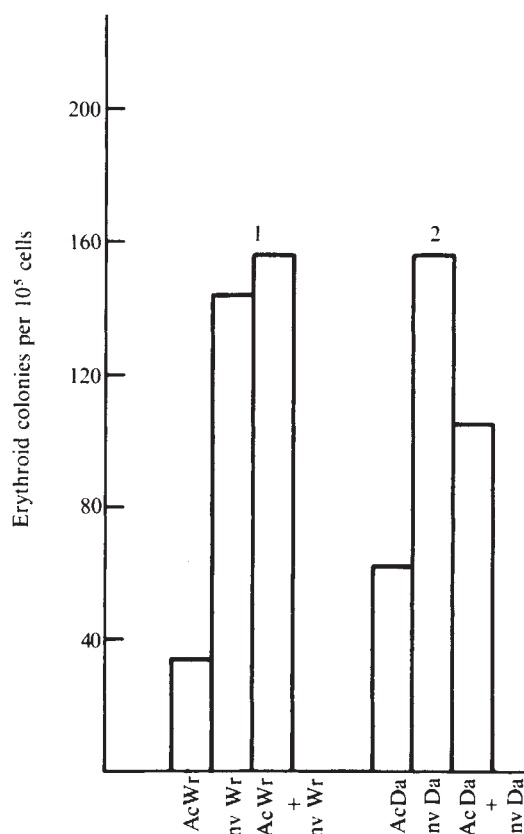


Fig. 2 Sera from two patients (Wr, Ba) obtained in the convalescent phase (Conv) of SPLV-related illness were assayed for their ability to neutralize the effect of the corresponding acute phase (Ac) serum on erythropoiesis. $150 \mu\text{l}$ of a 10^{-2} dilution of acute phase serum or convalescent phase serum, or a 1+1 mixture of the diluted sera, were added to 3×10^5 cells in a total volume of either $165 \mu\text{l}$ or $315 \mu\text{l}$ and incubated and plated as described in the legend for Fig. 1. The results are expressed as mean number of CFU-E derived colony counts per 10^5 nucleated cells plated as scored in replicate plates.

Table 1 Inhibition of erythropoiesis by SPLV

Expt 1 (SPLV-containing sera; source JH)	CFU-E per 10 ⁵ nucleated cells	(% Control)
SPLV-sera	1.5	(1)
Pre-heated SPLV-sera*	140	(123)
SPLV-sera + autologous serum†	132	(116)
Pre-heated SPLV-sera + autologous serum	128	(112)
Control	114	
Expt 2 (SPLV-containing sera; source Wr)		
SPLV-sera + anti-Ig 1:60	48	(12)
SPLV-sera + anti-Ig 1:100	128	(38)
SPLV-sera + anti-Ig 1:500	46	(10)
Anti-Ig	392	(120)
Control	338	(120)

Each experiment was performed with a different normal bone marrow donor, accounting for the difference in total number of CFU-E in control plates. In each experiment, SPLV-containing sera, diluted 10⁻², were added to 3 × 10⁵ cells in a total volume of 150 µl of Iscove's modification of Dulbecco's Eagle media: 2% fetal calf serum (IMDM) for 4 h at 4 °C, after which the cell suspension was added to complete tissue culture medium as described in Fig. 1 legend.

* SPLV-containing serum was heated at 56 °C for 30 min before mixing with bone marrow cells.

† Autologous human serum in a final dilution of 1:30 was added to the serum-cell mix before the 4 h incubation. In expt 2, SPLV-containing serum was incubated for 1 h with anti-human immunoglobulin (Miles) diluted in IMDM as shown at 4 °C, followed by incubation with bone marrow cells and plating as above.

Table 2 Effect on erythropoiesis of sera from a patient with hereditary spherocytosis in aplastic crisis

Sera	SPLV Ag +*	Anti- SPLV -	CFU-E per 10 ⁵ cells	BFU-E per 10 ⁵ cells	CFU-C per 10 ⁵ cells
Acute (5/7)	+	-	0 (0)	2 (4)	37 (86)
Convalescent (6/15)	-	+	102 (89)	43 (81)	52 (121)
Control	-	-	114	53	43

Dilutions (10⁻²) of sera from patient JH in IMDM were incubated with bone marrow cells from a healthy donor (DG) and cultured as described in the legend to Fig. 1. A control serum was similarly tested. Mean counts (two plates) and, in parentheses, % of control counts are shown. SPLV antigen and antibody were assayed by CIE.

* Titre 1:32.

sample. The preparation was free of IgG and IgM when tested in anti-human IgG- and IgM-containing gels (Melroy). The partially purified virus eliminated late erythroid colony formation, without markedly changing the inhibition of colony formation by BFU-E and CFU-C compared with the crude serum.

SPLV titres of the sera studied ranged from 1:8 to 1:128. No correlation between degree of inhibition of haematopoietic colony formation and titre was seen, suggesting that biological activity and antigenic titre of SPLV were not closely related. We noted a decrease in inhibitory activity of specimens in successive experiments without a change in antigen titre, and this may represent loss of virus viability with repeated freezing and thawing.

Convalescent sera available from the two patients following febrile illnesses, both with high anti-SPLV titres, were tested for inhibitory activity and ability to neutralize the suppressive effect of acute phase serum containing SPLV from the same patient (Fig. 2). In this experiment, the acute phase sera inhibited the formation of CFU-E derived colonies, expressed as colony number per 10⁵ nucleated cells plated, to levels of 20–40% of control values. In contrast, the convalescent sera showed little or no inhibitory activity. Mixtures (1:1) of the convalescent with the acute sera partially or completely eliminated the inhibitory effect of the acute phase serum alone.

Studies of the effects of antibody on haematopoiesis have generally used undiluted serum which can give a final concentration of over 20% of tissue culture volume^{13,14}. The effect of SPLV-positive sera occurred at a final dilution in tissue culture of 2 × 10⁻³, implying that the mechanism of inhibition was unlikely to be mediated by antibody. Heating of serum to 56 °C

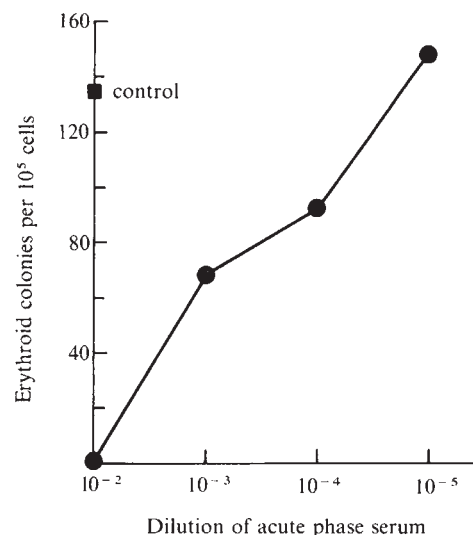


Fig. 3 Inhibition of CFU-E-derived erythroid colony formation by acute phase serum from the patient with hereditary spheroblastosis in aplastic crisis (J.H.). Bone marrow cells were incubated with serial 10-fold dilutions of the serum in IMDM and cultured as described in Fig. 1 legend. Results represent the mean colony count (two plates) per 10⁵ cells. The control value shown was obtained with a 10⁻² dilution of serum from the bone marrow donor.

for 30 min resulted in diminished inhibitory effect on erythropoiesis, but the addition of fresh serum from the bone marrow donor, as a source of complement, did not restore inhibitory activity. Preincubation of the serum preparations with anti-human immunoglobulin did not decrease inhibitory activity (Table 1). These results further argue against a complement- or antibody-mediated mechanism of action. Most parvoviruses resist heating to 56 °C, but some require the help of a second, heat-sensitive virus for replication¹⁵. The heat sensitivity of the SPLV-containing serum, which was also observed with other of these sera, suggests that SPLV may either be unusually heat labile or not an autonomous parvovirus.

In a subsequent experiment, sera from a patient (JH) with hereditary spherocytosis who had an aplastic crisis were studied. This 8-year-old boy developed severe anaemia with absolute reticulocytopenia during a febrile illness. The characteristics of the sera obtained during the acute and convalescent phases of the crisis are shown in Table 2. Only the acute specimen, in which SPLV antigen was detectable by CIE, markedly inhibited erythropoiesis *in vitro*. There was a striking difference between the level of inhibition of erythroid and myeloid colony formation, consistent with the predominant clinical effect of the viral infection on the erythroid lineage. As seen in Fig. 3, the effect of the acute phase serum on *in vitro* erythropoiesis was apparent to a dilution of 10⁻⁴.

These results provide evidence of a suppressive effect of a human virus on haematopoiesis *in vitro*, and indicate that the SPLV-associated aplastic crises of the haemolytic anaemias may be the direct result of virus cytotoxicity for erythroid progenitors in the bone marrow. This effect on erythropoiesis, while it may occur in all infected individuals, probably only becomes apparent when a coexisting haemolytic process causes rapid destruction of circulating red blood cells and greatly increases the daily requirement for erythrocyte production by the bone marrow. The reversal of the inhibitory effect on erythropoiesis of acute phase serum by serum containing antibody to the virus suggests that the abrupt recovery from aplastic crises is due to inactivation of virus. It is possible that other bone marrow failure syndromes are promoted by viral infection of haematopoietic progenitors, including stem cells. Transient erythroblastopenia and aplastic anaemia of childhood, both of which commonly follow a viral infection, may also be due to a viral effect on haematopoietic cells.

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Responses to the $H-2K^b$ mutant involve recognition of syngeneic Ia molecules

Ofra Weinberger, Ronald N. Germain* & Steven J. Burakoff

Division of Pediatric Oncology, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115, USA

* Laboratory of Immunology, NIAID, National Institute of Health, Bethesda, Maryland 20205, USA

Conventional antigens appear to be recognized by T lymphocytes only when associated with major histocompatibility complex (MHC) antigens. Using antigen-specific proliferation as a model for helper T lymphocytes, it has been demonstrated that $Ly1^+$ T cells recognize antigen presented in association with syngeneic Ia molecules^{1,2}. In contrast to responses to conventional antigens, however, a large number of studies have suggested that the stimulation of alloreactive $Ly1^+$ T cells, and helper T cells specific for allogeneic cytotoxic T lymphocyte (CTL) responses, involve the direct recognition of Ia alloantigens^{3–5}. For the generation of optimal allogeneic CTL activity it has been proposed that $Ly1^+$ T cells recognize allo-Ia antigens directly and provide help to pre-CTLs that respond to allo- $H-2K$ and/or D determinants. Thus, the $B6.C.H-2^{bm1}$ mutant ($bm1$, formerly referred to as $H21$), which is believed to consist of a substitution of two amino acids in the $H-2K^b$ antigen⁶, has presented a paradox, for it can stimulate strong mixed lymphocyte culture (MLC), graft versus host and CTL responses^{7,8} by T cells of $H-2^b$ haplotype mice in the apparent absence of any alloantigenic differences in the I region. We now present evidence that the stimulation of proliferative and helper T cells by the mutant $B6.C.H-2^{bm1}$ results from the $H-2K^b$ antigen being recognized in the context of syngeneic Ia determinants. Thus responses to both conventional antigens and allogeneic MHC gene products may proceed via the recognition of antigen in the context of self Ia molecules.

When C57BL/6 spleen cells were cultured with $B6.C.H-2^{bm1}$ stimulator cells in a one-way MLC, strong stimulation was observed (Table 1), as has been previously reported^{7,8}. To establish whether such stimulation results solely from the recognition of the mutant $H-2K^b$ antigen, as has been proposed, or whether, in fact, $H-2$ antigens are recognized in the context of syngeneic Ia determinants, a monoclonal anti-Ia antibody was added at the initiation of a C57BL/6 anti- $bm1$ mixed lymphocyte culture as a blocking reagent. Complete blocking of the stimulation was observed with M5/114.2, a monoclonal antibody with specificity for I-A^b. MKD6, a monoclonal antibody with irrelevant specificity to I-A^d, had no effect on stimulation.

Table 1 $B6.H-2^{bm1}$ induced MLC is blocked by monoclonal anti-Ia antibody

Responding cells	Stimulating cells	Specificity of antibody added	Incorporation of 3H -thymidine (c.p.m.)(±s.d.)
C57BL/6	—	—	3,021 (±197)
C57BL/6	$B6.C.H-2^{bm1}$	—	35,805 (±2,053)
C57BL/6	$B6.C.H-2^{bm1}$	I-A ^b	335 (±41)
C57BL/6	$B6.C.H-2^{bm1}$	I-A ^d	28,985 (±1,829)

1×10^6 C57BL/6 ($H-2^b$) spleen cells were cultured in flat-bottomed microtitre wells with 1×10^6 irradiated $B6.C.H-2^{bm1}$ ($H-2^{ba}$) spleen cells in 200 μ l of supplemented RPMI 1640 medium containing 0.5% normal mouse serum. Where indicated, 40 μ l of monoclonal antibody (Mab) were added at the start of the culture. Supernatants from rapidly growing cell cultures were used 3 to 4 days after fresh passage and were used for blocking studies at a final concentration of 20%. M5/114.2 is a rat Mab with specificity for I-A^b, I-A^d, I-E^d, I-E^k and I-A^q (ref. 15) and MKD6 is a murine Mab with specificity for I-A^d (ref. 16). Proliferation was measured on day 3 by pulsing the culture with 0.4 μ Ci 3H -thymidine (3H -TdR) for 6 h and measuring 3H -TdR uptake.

Table 2 Generation of CTLs specific for the $H-2K^b$ mutation is blocked by monoclonal anti-Ia antibody

Responding cells	Stimulating cells	Specificity of antibody added	^{51}Cr release	
			33:1	11:1
C57BL/6	$B6.C.H-2^{bm1}$ (5×10^6)	—	66	34
C57BL/6	$B6.C.H-2^{bm1}$ (5×10^6)	I-A ^b	61	49
C57BL/6	$B6.C.H-2^{bm1}$ (5×10^5)	—	61	50
C57BL/6	$B6.C.H-2^{bm1}$ (5×10^5)	I-A ^b	48	13
C57BL/6 + IL-2	$B6.C.H-2^{bm1}$ (5×10^5)	—	58	66
C57BL/6 + IL-2	$B6.C.H-2^{bm1}$ (5×10^5)	I-A ^b	64	63
C57BL/6	$B6.C.H-2^{bm1}$ (10^5)	—	64	38
C57BL/6	$B6.C.H-2^{bm1}$ (10^5)	I-A ^b	15	—1
C57BL/6 + IL-2	$B6.C.H-2^{bm1}$ (10^5)	—	56	41
C57BL/6 + IL-2	$B6.C.H-2^{bm1}$ (10^5)	I-A ^b	57	42

6×10^6 C57BL/6 spleen cells were cultured with irradiated $B6.C.H-2^{bm1}$ stimulator cells for 5 days in supplemented RPMI 1640 medium containing 10% fetal calf serum. Where indicated, the monoclonal antibody M5/114.2 was added at the initiation of culture. IL-2 was a partially purified supernatant from concanavalin A-stimulated rat splenocytes, as previously described¹⁷, and was added at the start of culture at a final concentration of 10%. After 5 days of culture, cells were collected and assayed in a 4-h ^{51}Cr release assay on $bm1$ lipopolysaccharide blasts at the indicated effector to target ratios. Data were calculated as % specific release¹⁸ and the spontaneous release of ^{51}Cr from the target cells was <22%.

The importance of syngeneic Ia determinants in the generation of CTL responses was also investigated. When 6×10^6 C57BL/6 responder cells were cultured with an equivalent number of $bm1$ stimulator cells (5×10^6), the addition of anti-Ia antibody to the culture did not block the generation of a response (Table 2). However, when a suboptimal number of stimulator cells was added to culture, a role for syngeneic Ia antigens became apparent: with 10^5 stimulator cells, the CTL activity was reduced from 64% to 15% by the addition of M5/114.2. Earlier we demonstrated⁹ that the involvement of Ia⁺ adherent accessory cells as well as $Ly1^+$ helper T cells in the generation of CTL responses to alloantigen is best demonstrated with limiting numbers of stimulator cells⁹. Thus, the inhibition of CTL responses by anti-Ia antibody could reflect blocking of helper T cells specific for alloantigen re-presented by Ia⁺ syngeneic accessory cells or alternatively could result

Table 3 The Ia antigens of splenic adherent cells are involved in presentation

Responding cells	Stimulating cells	Removal of Ia ⁺ cells	Accessory, splenic adherent, cells	Specificity of antibody added	Incorporation of ³ H-thymidine (c.p.m.)(±s.d.)	⁵¹ Cr release	
						13:1	3:1
C57BL/6	—	—	—	—	2,989 (±203)	0	0
C57BL/6	B6.C.H-2 ^{bm1}	—	—	—	23,789 (±2,111)	97	85
C57BL/6	B6.C.H-2 ^{bm1}	—	—	I-A ^b	4,651 (±293)	34	8
C57BL/6	B6.C.H-2 ^{bm1}	+	—	—	3,527 (±334)	9	0
C57BL/6	B6.C.H-2 ^{bm1}	+	C57BL/6 (10 ⁵)	—	7,713 (±596)	42	16
C57BL/6	B6.C.H-2 ^{bm1}	+	C57BL/6 (6×10 ⁵)	—	17,891 (±1,239)	75	51
C57BL/6	B6.C.H-2 ^{bm1}	+	C57BL/6 (6×10 ⁵)	I-A ^b	5,257 (±491)	0	0
C57BL/6	B6.C.H-2 ^{bm1}	+	bm1 (10 ⁵)	—	14,013 (±992)	79	54
C57BL/6	B6.C.H-2 ^{bm1}	+	bm1 (6×10 ⁵)	—	19,665 (±1,732)	84	65
C57BL/6	B6.C.H-2 ^{bm1}	+	bm1 (6×10 ⁵)	I-A ^b	4,907 (±505)	0	0

Where indicated, Ia⁺ cells were depleted by passage of spleen cells over nylon wool followed by treatment with monoclonal anti-Ia antibody (M5/114.2) plus complement (C). Splenic adherent cells were prepared as previously described^{19,20} by adherence to glass dishes at 37 °C for 2–3 h and subsequent removal with Versene buffer (Gibco).

from interference with the recognition of the antigen by the pre-killer cell. The inability to block stimulation with higher doses of stimulator cells may result from the inability of the antibody to block all determinants. Alternatively, at high stimulator cell numbers the cytotoxic T cell may be directly stimulated by the H-2K^{ba} molecule in a helper-independent manner or small numbers of allospecific helper cells for H-2K^{ba} may be induced.

A distinction between the helper T cell and the pre-killer cell could not be made in this case by fractionating the responding cell population with anti-Ly1 and anti-Ly2 antibodies because the phenotypes of the bm1 specific proliferating cell¹⁰ and cytotoxic cell¹¹ are both Ly1⁺, 2⁺, 3⁺. The requirement for accessory cells and T helper cells for these responses can be bypassed, however, by the addition of a T-cell derived helper factor (interleukin-2, IL-2) to the CTL cultures. Therefore, the site of action of the blocking antibody was investigated by the effect of the addition of IL-2 on the ability to block induction of a response. The results in Table 2 demonstrate that if a helper cell requirement is bypassed by the addition of IL-2 to the culture, the presence of anti-Ia antibody has no effect on the induction of a response. Thus, the H-2^{ba} mutation in the H-2K region of the bm1 appears to be recognized in association with syngeneic Ia determinants by the helper T cells, and not by the pre-killer cells, for the generation of a CTL response.

In an attempt to determine whether the blocking we observed occurs at the level of the splenic adherent cell and whether the alloantigen can be re-presented by splenic adherent cells of responder origin, mixing experiments were carried out. Both the responding and stimulating spleen cell populations were depleted of adherent cells by passage through nylon wool and treatment with anti-Ia antibody plus complement¹² and irradiated splenic adherent cells of either responder or stimulator origin, prepared by adherence to glass dishes^{13,14}, were added back at the initiation of culture. It was observed that whereas the depletion of Ia⁺ adherent cells completely abrogated both the proliferative and CTL responses, responsiveness could be reconstituted by the addition of splenic adherent cells of either responder or stimulator type (Table 3). The reconstitution of responsiveness with irradiated adherent cells, and the ability to block this reconstitution with M5/114.2 suggests that the Ia present on the splenic adherent cells is the target for the blocking antibody.

The proliferative response to an alloantigenic difference in the H-2K region appears to be similar to the response to a conventional antigen, that is, via recognition in the context of syngeneic Ia determinants. However, it was surprising that proliferation to H-2K incompatible stimulators is mediated by Ly1⁺2⁺ cells¹⁰ rather than by Ly1⁺2[−] cells as in other Ia antigen presenting systems^{1,2}. In Table 4 we demonstrate that a critical factor affecting the phenotype of the proliferating responder is whether the response is a primary or secondary MLC. As has

Table 4 Proliferating cells in secondary response to B6.C.H-2^{bm1} are Ly1⁺2[−]

Responding cells	Stimulating cells	Incorporation of ³ H thymidine (c.p.m.)(±s.d.)
C57BL/6	B6.C.H-2 ^{bm1}	22,397 (±1,736)
C57BL/6(anti Ly1 & C)	B6.C.H-2 ^{bm1}	7,327 (±495)
C57BL/6(anti Ly2 & C)	B6.C.H-2 ^{bm1}	6,434 (±468)
C57BL/6(anti Ly1 & C) and	B6.C.H-2 ^{bm1}	7,379 (±523)
C57BL/6(anti Ly2 & C)	B6.C.H-2 ^{bm1}	23,013 (±1,343)
C57BL/6 (2°)	B6.C.H-2 ^{bm1}	4,060 (±501)
C57BL/6 (2°)(anti Ly1 & C)	B6.C.H-2 ^{bm1}	18,191 (±1,517)
C57BL/6 (2°)(anti Ly2 & C)	B6.C.H-2 ^{bm1}	17,902 (±1,491)
C57BL/6 (2°)(anti Ly1 & C) and	B6.C.H-2 ^{bm1}	17,902 (±1,491)
C57BL/6 (2°)(anti Ly2 & C)		

C57BL/6 spleen cells were from naive mice or from mice primed subcutaneously to bm1 1 week earlier (2°). The responders were treated with either C, monoclonal anti-Ly1.2 plus C, or anti-Ly2.2 plus C. A total of 1×10⁶ responders were cultured with 5×10⁵ irradiated B6.C.H-2^{bm1} spleen cells.

been reported previously¹⁰, in a primary proliferative response to the H-2^{ba} mutation the response is mediated by Ly1⁺2⁺ lymphocytes. In a secondary response, however, the phenotype of the responding cell shifts to Ly1⁺2[−], just as the proliferating cells in secondary responses to conventional, soluble antigens are Ly1⁺2[−].

In conclusion, the results reported here demonstrate that although a difference in the H-2K region alone can apparently stimulate a strong proliferative as well as helper T-cell response, it does so not by being recognized directly but rather by being recognized in the context of syngeneic Ia determinants. These results, in part, help to clarify the unexpectedly strong proliferative and cytotoxic responses observed between the C57BL/6 strain and the bm1 mutant strain. In most model systems studied, proliferative and helper T cells are stimulated by Ia antigens, whether they are alloantigens or conventional antigens plus syngeneic Ia determinants. We have recently demonstrated that purified H-2K^a alloantigen incorporated into liposomes is recognized by helper T cells in association with syngeneic Ia determinants¹², indicating that allogeneic MHC gene products can be recognized in association with syngeneic Ia antigens in a manner similar to conventional, non-MHC antigens. Furthermore, the alloantibody response¹³, much like the allospecific CTL response¹⁴, has also been demonstrated to be restricted by I region products syngeneic to the responder. The data presented here demonstrate that this unifying concept can also be applied to responses stimulated by the bm1 mutant.

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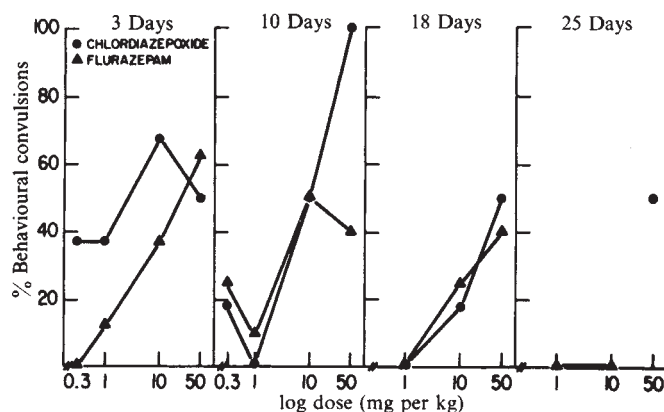


Fig. 1 The per cent of pups that showed behavioural convulsions at four different ages. The abscissa values are doses of chlordiazepoxide (●) or flurazepam (▲) given intraperitoneally in a volume of 1 ml per 100 g. Note that the lower doses of chlordiazepoxide and flurazepam were only given to the younger animals. None of the saline or diluent controls showed convulsions. Each data point represents six to eight subjects, and each subject was used once.

Effect of age on benzodiazepine-induced behavioural convulsions in rats

Gordon A. Barr

Division of Neuropsychopharmacology, Department of Psychiatry, Albert Einstein College of Medicine, Bronx, New York 10461, USA and Biopsychology Program, Department of Psychology, Hunter College of the City University of New York, New York, New York 10021, USA

Ted Lithgow

Biopsychology Program, Department of Psychology, Hunter College of the City University of New York, New York, New York 10021, USA

The benzodiazepines are a class of drugs used to alleviate anxiety. As such they constitute one of the most commonly prescribed compounds¹, due in part to their efficacy and safety. The physiological effect of these drugs is probably through interactions with a low affinity benzodiazepine binding site² and two (types 1 and 2) higher affinity sites³⁻⁵. The ontogenesis of these latter two binding sites in the rat differs, with the type 2 binding site being predominant at birth and the type 1 binding site increasing in number after the second week after birth⁶. The differential development of these two receptor types is important because the immature organism may have different physiological and behavioural responses from the adult. Here we demonstrate an important difference: that a prototypic benzodiazepine, chlordiazepoxide, and a water-soluble benzodiazepine, flurazepam, produce behavioural convulsions in the preweanling rat. The convulsions are antagonized by the benzodiazepine blocker Ro-15-1788. The triazolopyridine CL-218872, specific to the type 1 receptor, does not share this action. We suggest that this paradoxical convulsant effect of chlordiazepoxide and flurazepam is due to activation of the type 2 receptor in the absence of the type 1 receptor in the immature rat.

Long Evans hooded rat pups born to breeding pairs in our colony were maintained at $22 \pm 2^\circ\text{C}$ on a 12-h on/12-h off light cycle. Pups were designated age 0 at birth and tested at 3, 10, 18 and 25 days of age. Flurazepam was dissolved in physiological saline, and chlordiazepoxide (Hoffman-LaRoche) was dissolved in the diluent supplied by the manufacturer. Because of the possible effects of the diluent by itself or in interaction with

chlordiazepoxide, control experiments were performed in which the drug was dissolved in physiological saline, or the diluent alone injected. In other experiments, CL-218872 was dissolved in saline to which a slight amount of ethanol was added. Ro-15-1788 was suspended in saline, four drops of ethanol and four drops of Tween 80. In all experiments the drugs were administered to the pups intraperitoneally in a volume of 1 ml per 100 g body weight. Behavioural observations were begun immediately after drug treatment in a room maintained at 26°C . Disposable syringes and needles (26 gauge) were used to eliminate sources of error attributable to benzodiazepine contamination¹⁰. Behavioural convulsions were defined by either unilateral tonic-clonic movements or bilateral tonus in association with at least one or more of a constellation of convulsion-related behaviours, including straub tail, jaw tremor, full body spasm, limb paralysis, loss of righting reflex or 'swimming' movements (only in 3- and 10-day-old animals).

The data for chlordiazepoxide and flurazepam are shown in Fig. 1. The percentage of animals that convulsed decreased with increasing age of the pup, and younger pups convulsed even at very low chlordiazepoxide doses. These lower doses were within the range given to rats in paradigms used to assess the anxiolytic properties of these drugs¹¹. The latency to show convulsions was less than 10 min and consistent across ages. Chlordiazepoxide dissolved in saline was as effective in producing convulsions; the Roche diluent or saline never produced convulsions, although a few pups exhibited one or more of the convulsion-related behaviours following the diluent. The decrease in the number of pups showing convulsions at the highest dose of chlordiazepoxide and flurazepam for 3- and 10-day-olds respectively, was unexpected. Flurazepam was less effective than chlordiazepoxide in producing seizures in 3-day-old animals, but 10-day-olds showed almost identical response curves. CL-218872 did not produce convulsions (0/6 and 0/5 in 3- and 10-day-olds, respectively, for 10 mg per kg; 50 mg per kg was lethal).

Behavioural convulsions may have been the result of non-specific pharmacological effects in the developing neonate. To address this issue, two additional groups of 10-day-olds were pre-injected with a putative benzodiazepine blocking agent, Ro-15-1788 (10 mg per kg), 15 min before injection of the ED₅₀ of chlordiazepoxide (10 mg per kg) or flurazepam (10 mg per kg). The Ro-15-1788 reduced the proportion of animals that convulsed after chlordiazepoxide (0/8, $P = 0.038$, Fisher's test) and flurazepam (1/8, $P = 0.13$).

We do not know why anticonvulsants in adults produce seizures in an immature animal. However, one interpretation

is the selective activation of one receptor relative to another. There is a striking inverse correlation between the development of type 1 benzodiazepine receptors and the convulsant properties reported here. Benzodiazepines seem to bind uniformly to both types of receptor. Conversely, triazolopyridazines bind to the type 1 binding site with considerably higher affinity than to the type 2 site¹². Although active as anxiolytic and anticonvulsant agents, the triazolopyridazines do not produce typical side effects such as sedation or ataxia, associated with the benzodiazepines¹³. The present behavioural data suggest that binding to the early-appearing type 2 receptor results in behavioural convulsions as well as typical side effects. We submit that there may be at least two actions of benzodiazepines in brain, one which facilitates and one which inhibits seizures, and that the inhibition normally overrides facilitation. In the neonate the inhibitory system is not fully developed and therefore is incapable of this protective mechanism. As the type 1 receptor or its components develop, the ability to induce seizures is lost. That the type 1-specific CL-218872 compound did not produce seizures supports this contention. Alternatively, of course, it is possible that other physiological systems affected by benzodiazepines change as the animal matures, such that there are qualitative differences between these systems in the pup and the adult.

Although it is difficult to generalize across species, brain growth rates suggest that the postpartum rat pup is equivalent developmentally to the third trimester human infant¹⁴. The benzodiazepines readily cross the placental barrier and there is some evidence that the concentration of these drugs is higher in the fetus than in the mother¹⁵. However, the known toxicological and teratogenic risks are slight, if indeed they exist at all. Diazepam administered to women in labour can cause hypothermia and hypotonia in the infant¹⁶, although both effects are short-lived. Diazepam in mother's milk can cause electroencephalograph changes in infants¹⁷. The only reported teratological property is the possible increased risk of cleft palate and even this risk is small and unreliable¹⁷⁻¹⁹. Moreover, despite the widespread use of benzodiazepines, we are aware of only one report of chlordiazepoxide-related postnatal seizures, and these occurred during benzodiazepine withdrawal²⁰.

Future work will attempt to determine important neural substrates of convulsant-inducing actions of benzodiazepines in neonates. However, the surprising findings here underscore the importance of different actions of pharmacological agents in the neonate and the adult.

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Histamine and noradrenaline decrease calcium-activated potassium conductance in hippocampal pyramidal cells

Helmut L. Haas & Arthur Konnerth*

Neurochirurgische Universitätsklinik, CH-8091, Zürich, Switzerland

Ample evidence exists for histaminergic¹ and noradrenergic^{2,3} projections to the hippocampus. Both amines exert neurotransmitter or modulator actions on principal neurones in the CA 1 and in the dentate area. A number of mechanisms have been proposed for these actions, including increased potassium conductance⁴, increased chloride conductance and electrogenic pump stimulation^{5,6}, and reduction of the anomalous inward rectification⁵. Action potentials, and particularly bursts of spikes, in CA 1 pyramidal cells, are followed by an afterhyperpolarization (AHP) which consists of two components⁷. The late AHP depends on a calcium-activated potassium conductance gK^+ (Ca^{2+})⁸⁻¹³, and has recently been shown to be increased by dopamine¹⁴. We report here a rapid and reversible decrease of the late AHP component following a burst of sodium spikes or a calcium spike, during perfusion with micromolar concentrations of histamine and noradrenaline. This effect is mediated by H_2 receptors and β -receptors, respectively, and occurred in the absence of changes in the calcium spike. By such a mechanism histamine and noradrenaline can profoundly potentiate the excitatory impact of depolarizing signals.

Transverse slices from the hippocampi of 24 rats were incubated at 32 °C in a perfusion chamber¹⁵. Micropipettes with resistances of 30-100 M Ω for intracellular recording from CA 1 pyramidal cells were filled with 4 M K^+ acetate or 3 M K^+ chloride. Neurone resting potentials were more negative than -60 mV, and action potentials greater than 80 mV; input resistances were approximately 40 M Ω . Tetrodotoxin (TTX, 10^{-6} g ml⁻¹) was added to the perfusion fluid in order to study slow, presumably calcium, spikes^{16,17} and their AHPs in most experiments. AHPs were elicited before and during drug action by the injection of positive current (0.2-2 nA, 20-200 ms, 0.05 or 0.1 Hz, using the same depolarizing current, or a current producing the same number of spikes). Conductance was determined from negative pulses of the same size. When changes in polarization occurred current injection was used to allow testing of AHPs at the same voltage levels. AHPs were averaged from four sweeps with a duration of 5 s. Drug solutions were freshly prepared and added to the perfusion fluid to final concentrations of 10^{-7} - 10^{-4} M. Most cells were held in good condition for several (up to 9) hours so that all four amines (histamine, noradrenaline, dopamine and serotonin) and several antagonists could occasionally be tested on the same neurone.

The AHP following a train of action potentials or a calcium spike elicited by depolarizing current injection consisted of two components^{7,10,11}. The late AHP had a duration of 1-3 s and was blocked in contrast to the early component, in low Ca^{2+} (0.2 mM), high Mg^{2+} (4 mM) solution (nine cells), suggesting that only the late AHP depends on a gK^+ (Ca^{2+}). When histamine (10^{-6} M, nine cells in normal solution, seven cells in the presence of TTX) was added to the perfusion medium this late

* Permanent address: Max Planck Institute for Psychiatry, D-8000, München 40, FRG.

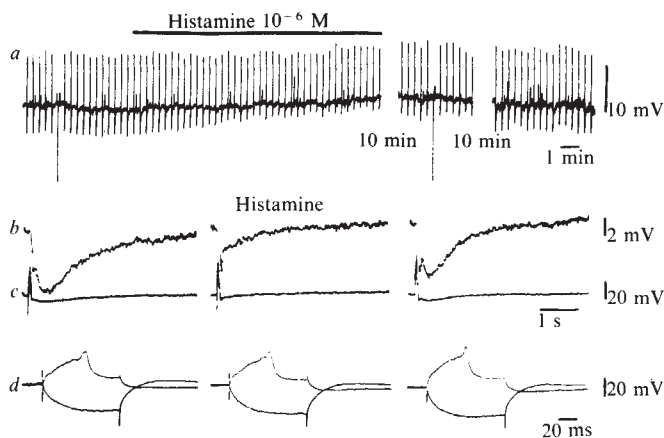


Fig. 1 The effect of histamine on membrane potential, conductance and AHPs after a slow (Ca^{2+}) spike in TTX-containing media. *a*, Pen recordings illustrating the membrane potential, Ca^{2+} spikes (upward deflections), and slow AHPs (downward deflections, except the two larger ones which mark the injection of negative current) evoked every 20 s by a +1 nA, 100 ms current pulse. Histamine was present in the perfusion medium during the time indicated by the bar above the trace. *b*, *c*, Traces showing the marked reduction of the late, but not the early, component of the AHP by histamine. *b*, Averaged trace (four sweeps); note difference in scale between *b* and *c*, the Ca^{2+} spike is out of scale in *b*. *d*, Superimposed voltage deflections caused by ± 1 nA current injection before, during and after perfusion with histamine.

AHP and its associated conductance were always markedly reduced, while the early AHP was unaffected (Fig. 1*b*, *c*). The membrane potential was usually depolarized by a few mV ($2.5 \text{ mV} \pm 1.5 \text{ s.d.}$), and the resting membrane conductance was not significantly changed (Fig. 1*a*, *c*). Spontaneous firing in preparations not treated with TTX was increased from below 1 to up to 10 spikes per s. The frequency of spontaneous synaptic events was also markedly enhanced, as could be seen particularly clearly in three cells recorded with K^+ chloride-filled electrodes, in which the number of spontaneous depolarizing inhibitory postsynaptic potentials (i.p.s.ps)^{18,19} was increased about three-fold by 10^{-6} M histamine. The calcium spikes observed in TTX poisoned preparations were sometimes reduced in size by higher amine concentrations (10^{-5} M), but the reduction in the AHP was often seen in the absence of any changes in the size and threshold of Ca^{2+} spikes (Fig. 1*d*). The same results were obtained irrespective of the electrodes (K^+ acetate or K^+ chloride) used for the recording. The action usually began within approximately 2 min after the addition of histamine to the perfusion fluid, reached a maximum in approximately 10 min, and began to recover approximately 5 min after washout. Complete recovery was seen in three cells within 30–120 min.

Noradrenaline and isoprenaline produced the same actions described above for histamine in all eight cells tested with the same dose range as histamine. The noradrenaline (10^{-5} M), but not the histamine (10^{-6} M), effect was blocked by the β -antagonist propranolol (2×10^{-5} M), whilst the H_2 antagonists metiamide and cimetidine (2×10^{-6} M) completely blocked or prevented the histamine, but not the noradrenaline, action. The H_1 antagonist mepyramine was ineffective against both amines at 10^{-5} M. These amine antagonists had no significant effect when given alone. The H_2 agonist impromidine (four cells) mimicked the actions of histamine in similar concentrations, whilst thiazoethylamine, an H_1 agonist, was ineffective at 10^{-4} M (three cells). The onset of the antagonist actions was considerably slower than the onset of the histamine and noradrenaline actions (5–10 min, as against 1–3 min). Serotonin (10^{-5} M) applied to two cells on which histamine and noradrenaline had typical effects caused a slight hyperpolarization and

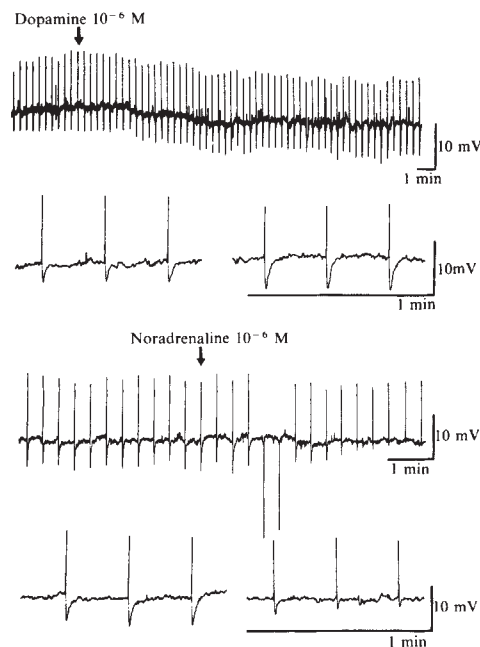


Fig. 2 Actions of dopamine and noradrenaline on membrane potential and AHPs in a TTX-containing medium. Dopamine and noradrenaline were added to the perfusion fluid at the time indicated by an arrow. Upward deflections are Ca^{2+} spikes, downward deflections are AHPs. Histamine had the same effect as noradrenaline 2 h earlier on this cell.

depression of firing, whilst the late AHP was increased by 30%. Dopamine (10^{-6} M) hyperpolarized four neurones by 2.2 mV ($\pm 0.6 \text{ s.d.}$) and increased the AHP, confirming the observation of Benardo and Prince¹⁴; on five cells dopamine (10^{-6} – 10^{-5} M) was ineffective. At 10^{-4} M (three cells) it had similar effects to those described here for histamine and noradrenaline. At this concentration dopamine may well be interacting with other amine receptors. Figure 2 shows chart records of Ca^{2+} spikes with their AHPs before and during the actions of dopamine and noradrenaline.

Histamine and noradrenaline have both been reported to produce a hyperpolarization associated with a modest conductance increase in CA 1 pyramidal cells when applied locally^{4,6}. With restricted local administration by iontophoresis or pressure ejection from fine pipettes histamine caused a hyperpolarization more often than a depolarization⁴. Larger drops on the slice surface lead more frequently to depolarizations^{4,20}. Hyperpolarizations but not depolarizations were also seen in high Mg^{2+} solutions, and in TTX poisoned preparations. They were thus direct effects on the pyramidal cell membranes, and were suggested to depend on a K^+ conductance mediated by H_2 receptors, which may be relatively small in number and spatially restricted⁴. The present findings indicate that another, depolarizing, action is more prominent when the amine is present in the perfusion fluid and influencing the whole cell. The depolarization caused by histamine and noradrenaline may be due to blockade of a tonic Ca^{2+} -dependent K^+ conductance. Therefore histamine and noradrenaline cause a reduction in $g\text{K}^+(\text{Ca}^{2+})$ together with an increase in the conductance of either Cl^- or K^+ . The $g(\text{Cl}^-)$ increase may be explained by our observation of the increased number of synaptic potentials. These are probably the result of an enhanced firing of inhibitory GABAergic interneurons. The exact distribution of receptors mediating the different actions is not known, but the difference between the effects of perfused and locally administered amines indicates a more general distribution of receptors mediating the reduction of $g\text{K}^+(\text{Ca}^{2+})$. A simultaneous increase and decrease of K^+ currents has been shown to be caused by some peptides in a snail neurone²¹. Such a dual action may be the

basis for the occasional excitatory or mixed responses of cortical neurones to ionophoretically applied histamine and noradrenaline^{22,23}. It also explains the minimal or lacking effect on net membrane conductance.

Another clue to the difference between local and bath application may be found in the report of Krnjević *et al.*²⁴ describing a similar action of noradrenaline and dopamine (depolarization and depression of AHPs) and serotonin (enhancement of AHPs) after injection of these amines into spinal motoneurones. It is therefore possible that the perfusion allows enough amine to enter the cells to reduce the available Ca^{2+} concentration, and consequently gK^+ (Ca^{2+}), by enhancing Ca^{2+} sequestration. A second messenger may be involved in this step, whether or not the amines enter the cells. The delayed onset of effects of antagonists lends further support to the idea of intracellular action. Both noradrenaline and cyclic AMP have been found to increase population spikes in hippocampal slices²⁵; this can be explained by the present findings as strong excitations are normally self-restricting as a result of gK^+ (Ca^{2+}) activation²⁶. Interestingly histamine and noradrenaline, but not dopamine or serotonin, increase cyclic AMP levels in hippocampal slices (refs 1, 27–29, and E. T. Browning, personal communication).

The AHP reduction in the absence of any detectable change in the Ca^{2+} spikes is also consistent with a stimulating effect of the amines on the intracellular Ca^{2+} sequestering system; alternatively, only the gK^+ component of gK^+ (Ca^{2+}) may be blocked. Anomalous rectification, which is probably due to an inward Ca^{2+} - Na^+ current³⁰, has been shown to be reduced by noradrenaline⁵. This finding seems to be unrelated to the one we describe here in normal and TTX-containing media, as anomalous rectification is blocked, or even reversed, by TTX (ref. 30 and our own unpublished observations). Both effects enhance the signal to noise ratio of excitatory transmission, an action previously suggested by others^{5,31,32}. These new and specific amine effects allow a powerful potentiation of the rapid information transfer at cortical synapses. In a report published after submission of this paper, Madison and Nicoll²⁶ showed that noradrenaline and cyclic AMP block the slow AHP and greatly enhance the responses of pyramidal cells to depolarization. This block of accommodation leads to a profound augmentation of excitatory signals.

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Chloroquine diverts ACTH from a regulated to a constitutive secretory pathway in AtT-20 cells

Hsiao-Ping Moore, Barry Gumbiner & Regis B. Kelly

Department of Biochemistry and Biophysics,
University of California, San Francisco, California 94143, USA

AtT-20 cells, a mouse pituitary line, externalize a viral membrane glycoprotein and the precursor of ACTH constitutively, that is, rapidly without storage or regulation¹. They also have a regulated pathway in which they cleave the precursor to mature hormones, ACTH and β -endorphin², store them in secretory granules³ and discharge them only in the presence of a secretagogue. An analogy exists for newly synthesized lysosomal enzymes which are either delivered to the lysosome or secreted from the cell^{4,5}. Targeting to the lysosomes may require a low pH step, since chloroquine causes the enzymes to be secreted from the cell^{5,6}. Here we show that chloroquine (200 μM) also appears to block the storage of newly synthesized ACTH in secretory granules and instead diverts it to the outside of the cell via the constitutive pathway. Chloroquine has no effect on the constitutive pathway and does not block the exocytosis of pre-packaged ACTH. Thus like lysosomal enzymes, peptide hormones are not sent to their correct destinations in the presence of chloroquine, but are diverted instead to a constitutive pathway that is chloroquine-insensitive.

Treating AtT-20 cells with chloroquine blocks intracellular storage and enhances the basal secretion of newly synthesized ACTH. To label the newly synthesized ACTH we pulse-labelled AtT-20 cells for 30 min with ³⁵S-methionine then chased for 2 h. Precursor and mature ACTH were identified by immunoprecipitation with antibody to mature ACTH. The effect of 200 μM chloroquine on the distribution of pulse-labelled ACTH between cells and culture medium after 2 h of chase is shown in Table 1. Because of the extensive constitutive release of precursor hormone by this cell line¹, untreated cells secrete 53% of their labelled ACTH molecules during the chase period. Most of the 47% remaining in the cells, however, is stored within secretory granules in the form of mature ACTH (molecular weights 4,500 and 13,000 (4.5 and 13 K))³. In the presence of chloroquine, only 17% of the ACTH molecules labeled during a pulse is retained within the cells after a 2-h chase. As shown below, little of this is in the form of mature ACTH (Fig. 3). Thus chloroquine treatment diverts newly

Table 1 Chloroquine enhances basal secretion of newly synthesized ACTH

	–Chloroquine	+Chloroquine
Total ACTH forms remaining in cell after 2-h chase	47	23*
Amount released during chase	53	112
Total synthesized	100	135

Confluent dishes (10-cm diameter) of AtT-20 cells were pulse-labelled with ³⁵S-methionine (0.4 mCi per dish) for 30 min and chased for 2 h. Chase media and cell extracts were collected, immunoprecipitated with an anti-ACTH (1–39) antiserum, and electrophoresed on SDS polyacrylamide gels¹. The various forms of labelled ACTH were quantitated by scanning the fluorograms with a densitometer and correcting for the number of methionine residues in each form. Numbers shown are the sum of all ACTH forms in each sample in arbitrary units, with total in the control set at 100. Chloroquine was added to the experimental dishes 1 h before radiolabelling and maintained at a concentration of 200 μM throughout the experiment.

* Note that the recovery of ACTH is higher in chloroquine (135%) than in control samples. Only 17% of the ACTH made is stored.

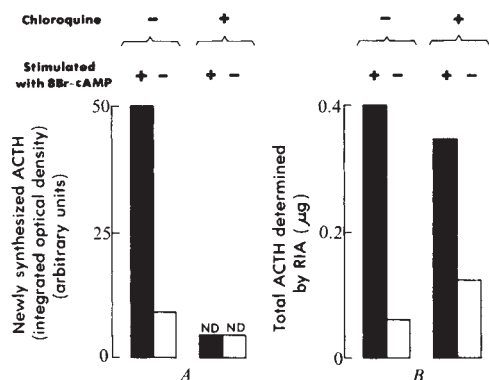


Fig. 1 Chloroquine blocks both basal and stimulated release of newly synthesized 4.5 K ACTH. AtT-20 cells were treated with chloroquine, radiolabelled with ^{35}S -methionine and chased for 1 h. 8-Bromo cyclic AMP 5 mM was then added to the medium during the second hour of chase and one-tenth of the samples were immunoprecipitated and analysed as described in Table 1. **A**, Labelled 4.5 K ACTH which was recovered in the culture medium during the second hour of chase. Fluorograms were exposed for 2 days and densitometer scans were quantitated by weighing areas under each peak. **B**, Total ACTH in the same medium determined by the radioimmunoassay described previously³. ND, not detected.

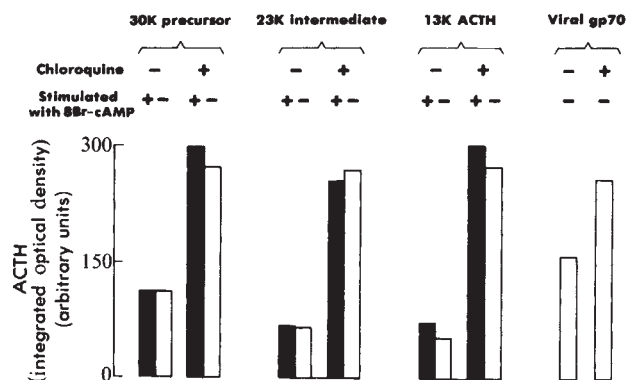


Fig. 2 Chloroquine does not block constitutive secretion. AtT-20 cells were pulse-labelled and chased as in Fig. 1. Radioactivity in the 30 K precursor ACTH, the 23 K biosynthetic intermediate of ACTH, and the 13 K glycosylated ACTH secreted during the second hour of chase was measured spectrophotometrically as described in Table 1 and Fig. 1. Viral gp70 in the medium was quantitated by immunoprecipitating one-fourth of the medium with an anti-gp70 antiserum. Results obtained from cells treated and untreated with bromo cyclic AMP and/or chloroquine are shown.

synthesized ACTH from intracellular storage within secretory granules to the outside of the cell.

Knowing that intracellular storage of ACTH occurs via the regulated pathway¹, we suspected that chloroquine was preventing the entry of ACTH into the regulated pathway. We used the mature 4.5 K ACTH as a marker for the regulated pathway because storage granules contain only mature ACTH³ and because the release of mature but not precursor ACTH is stimulated by noradrenaline or bromo cyclic AMP^{1,7}. Figure 1A shows that 200 μM chloroquine strongly inhibits both the basal and the bromo cyclic AMP dependent release of newly synthesized 4.5 K ACTH. The lack of stimulation by bromo cyclic AMP during chloroquine treatment is not due to an inhibition of exocytosis or stimulus secretion coupling. In the same experiment the release of unlabeled ACTH (measured by radioimmunoassay) that was already stored in secretory granules was stimulated by bromo cyclic AMP (Fig. 1B). We conclude that the regulated pathway is inhibited by chloroquine at some step prior to exocytosis. The enhanced secretion of newly synthesized ACTH during chloroquine treatment must therefore occur via some other pathway, perhaps the constitutive one.

If newly synthesized ACTH is rapidly released from AtT-20 cells by the constitutive pathway during chloroquine treatment (Table 1) then constitutive protein secretion must be insensitive to chloroquine. To test this prediction we examined the externalization of an envelope glycoprotein (gp70) of a murine leukaemia virus endogenous to AtT-20 cells since this protein is normally released by the constitutive pathway¹. The release of newly synthesized gp70 is not blocked by 200 μM chloroquine (Fig. 2). Also, the 30 K precursor ACTH and the 23 K intermediate, which are found in the medium as a result of constitutive secretion by AtT-20 cells¹, are released in the presence of chloroquine (Fig. 2). The secretion of other proteins by the constitutive pathway (H.P.M. *et al.*, manuscript in preparation) is also not affected by chloroquine (data not shown). ACTH molecules secreted in the presence of chloroquine are glycosylated normally in the Golgi apparatus. The secreted molecules contain complex type oligosaccharides which are acquired in the Golgi apparatus⁸, by the criterion of resistance to digestion with endoglycosidase-H^{9,10} (data not shown). We therefore conclude that the processing and secretion by constitutive pathway of AtT-20 cells are spared by chloroquine treatment.

The absence of 4.5 K ACTH secretion during chloroquine treatment is accompanied by an inhibition of 4.5 K ACTH

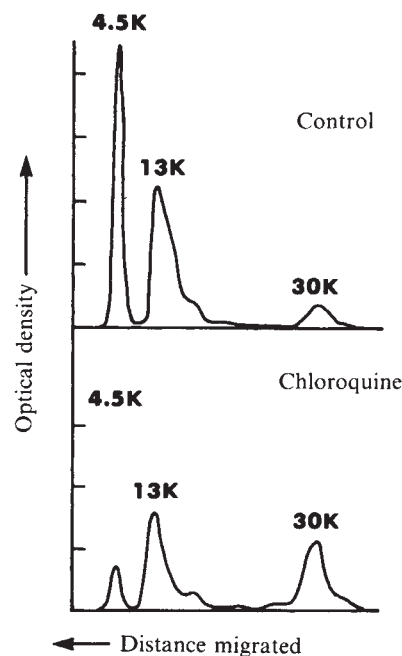


Fig. 3 Chloroquine prevents the accumulation of 4.5 K ACTH in cells. Radiolabelled cells in Fig. 2 were extracted at the end of 2 h of chase, immunoprecipitated with anti-ACTH antisera and analysed on SDS-polyacrylamide gels. Shown are the densitometer scans of SDS polyacrylamide gels of ACTH remaining in cells after 2 h of chase.

accumulation in cells. As shown in Fig. 3, treated cells contain less 4.5 K ACTH than the control cells. A possible explanation for the lack of appearance of 4.5 K ACTH is that chloroquine prevents the delivery of precursor ACTH to the secretory granule, which contains the specific proteases¹⁵. Together these data indicate that newly synthesized ACTH does not enter the regulated pathway in the presence of chloroquine but instead is diverted to the constitutive pathway, which is unaffected.

An unexpected observation is that chloroquine also enhances the core glycosylation of precursor ACTH, resulting in a greater proportion of glycosylated ACTH forms. For example the ratio of 13 K ACTH (a glycosylated form of 4.5 K ACTH) to 4.5 K ACTH is two- to threefold higher in chloroquine than in controls (Fig. 3). Because chloroquine increased the ratio of 13 K to 4.5 K ACTH, we should consider the possibility that 13 K

rather than 4.5 K ACTH is secreted by the regulated pathway in the presence of chloroquine. However, Fig. 2 shows that bromo cyclic AMP does not stimulate the release of 13 K ACTH in chloroquine-treated cells. Also, note that the release of the precursor forms (30 K and 23 K) is not enhanced in response to bromo cyclic AMP (Fig. 2), showing that they are also not being packaged in secretory granules instead of the 4.5 K form.

There are at least two examples in which a low pH step, blocked by lysosomotropic drugs, might be involved in targeting proteins, the delivery of newly synthesized lysosomal enzymes to the lysosome, and the dissociation of certain receptor ligand complexes during receptor mediated endocytosis^{11,12}. Although chloroquine is not a highly specific drug, it is possible that it exerts its effects on ACTH storage and secretion by AtT-20 cells in a similar way. Although there is no direct evidence for

a low pH inside ACTH-containing secretory granules, other secretory granules which contain peptides do have an acidic interior^{13,14}. Furthermore, a recently discovered enzyme which cleaves precursor ACTH and is associated with isolated secretory granules is activated by low pH¹⁵. Packaging of ACTH into secretory granules may be another example in which a low intraorganelle pH is required for correct sorting of protein molecules^{11,12}.

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Light-chain phosphorylation controls the conformation of vertebrate non-muscle and smooth muscle myosin molecules

Roger Craig*, Robin Smith & John Kendrick-Jones

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

Phosphorylation of the 20,000-molecular weight (M_r) light chains of vertebrate non-muscle (thymus) and smooth muscle (gizzard) myosins regulates the assembly of these myosins into filaments *in vitro*^{1,2}. At physiological ionic strength and pH, nonphosphorylated smooth muscle and non-muscle myosin filaments are disassembled by stoichiometric levels of MgATP, forming species having sedimentation coefficients of ~11S (range 10–12S^{1,3,4}; myosin monomers in high salt sediment at 6S). When the 20,000 (20K)- M_r light chains on these 11S myosin species are phosphorylated by the light-chain kinase/calmodulin- Ca^{2+} complex, the inhibitory effect of the light chains on filament formation is removed and the myosins reassemble into filaments which are stable in MgATP^{1,2,5,6}. It was originally suggested that the 11S myosin species was a dimer^{1,3}, previously suggested as a building block for smooth muscle and non-muscle myosin filaments^{7,8}. It has since been shown, however, that 11S smooth muscle myosin is monomeric^{4,9} and has a folded conformation^{4,10} rather than the extended shape characteristic of monomeric myosin in high salt^{11,12}. Here we show that 11S non-muscle myosin is also folded and that phosphorylation of the 20K- M_r light chains of both vertebrate non-muscle (thymus) and vertebrate smooth muscle (gizzard) myosins causes these folded 11S molecules to unfold into the conventional extended monomeric form, which is able to assemble into filaments.

When MgATP is added to nonphosphorylated vertebrate non-muscle (thymus) or smooth muscle (gizzard) myosin filaments at physiological ionic strength (0.15 M NaCl, pH 7.0) the filaments rapidly disassemble into ~11S myosin molecules^{1,3,4}. When these molecules are rotary-shadowed and examined under the electron microscope, they are seen to have a folded structure (Figs 1a, 2a, 4a). They are folded twice, at two hinge regions situated approximately one-third and two-thirds along the length of the tail so that the tail region is

Table 1 Effect of light-chain phosphorylation on the conformation of gizzard and thymus myosin molecules

Time points	Circled numbers (in Fig. 3)	Estimates of numbers of molecules Folded (%)	Extended (%)
Gizzard myosin			
11 min	1	70–80	20–30
17 min	2	30	70
19 min	3	20	80
22 min	4	10	90
34 min	5	10	90
Thymus myosin			
16 min	1	95	5
19 min	2	80–90	10–20
22 min	3	75–80	20–25
24 min	4	70–75	25–30
25 min	5	60–70	30–40
31 min	6	40	60

The estimates of numbers of folded and extended molecules are expressed as percentages of the total number of free molecules. Aliquots were taken from the assembly assays at the points indicated by the circled numbers in Fig. 3 and were rotary-shadowed. The corresponding times in these assays are also shown so that the estimates of folded and extended molecules can be compared with the level of light-chain phosphorylation shown in the polyacrylamide gels of Fig. 3. The percentages of folded and extended molecules are estimates made by visual scanning of 30 or more droplets of molecules from each spraying. There was some variation in the number of folded molecules between droplets and the percentages shown represent a weighted estimate. Although exact counts were not made, these approximate percentages were reproduced with both gizzard and thymus myosins in six experiments in each case and for at least three different preparations of each myosin. One would not expect a perfect correlation between the state of phosphorylation of these myosins and their conformations, because as the light chains are phosphorylated the molecules unfold and then assemble into filaments. (This is seen as a decrease in the total number of free molecules observed and an increase in the number of filaments as the assay proceeds.) The estimates of folded and extended molecules will thus tend to be biased towards the folded state.

divided into three segments. The number of folded molecules observed varies but we estimate that it is generally >95% in the case of thymus myosin and >80% for gizzard myosin (Table 1). Onishi and Wakabayashi¹⁰ observed a similar arrangement of the tail in their folded 10S gizzard myosin molecules (see legend to Fig. 4a).

When the folded thymus and gizzard myosin molecules are incubated with gizzard myosin light-chain kinase, calmodulin and Ca^{2+} , the 20K- M_r light chains are rapidly phosphorylated (Fig. 3). Light-chain phosphorylation is accompanied by a

* Present address: Department of Anatomy, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, Massachusetts 01605, USA.

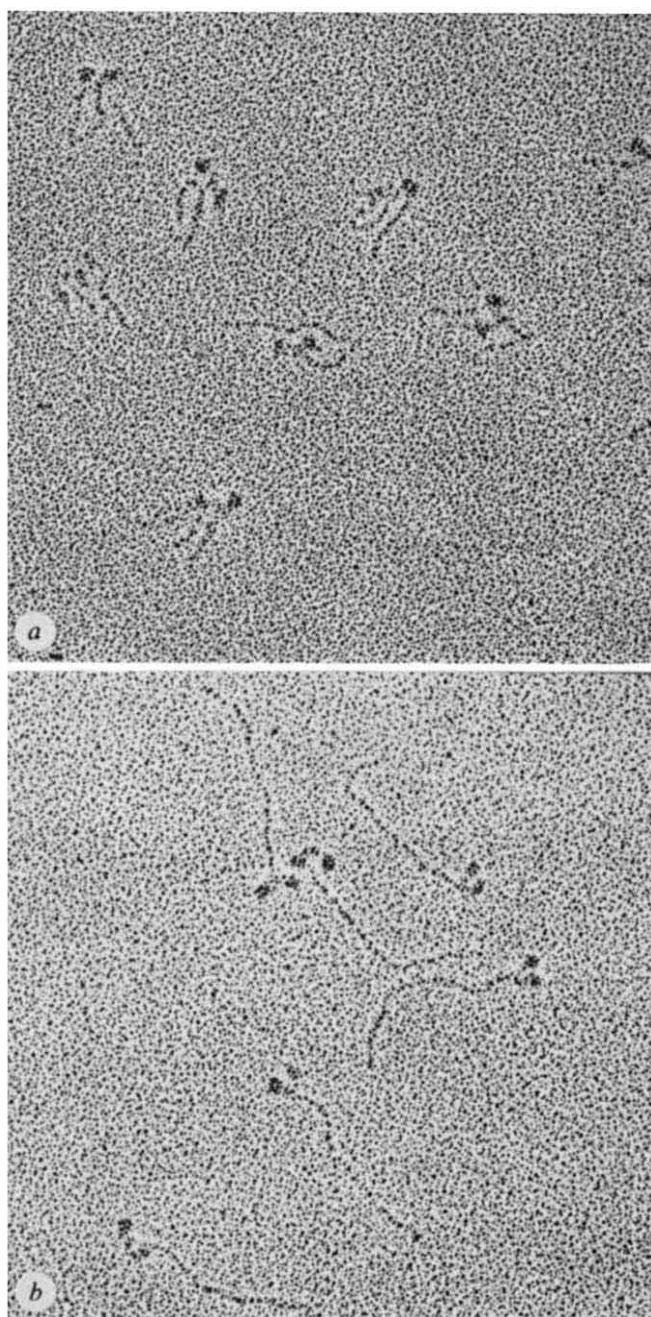
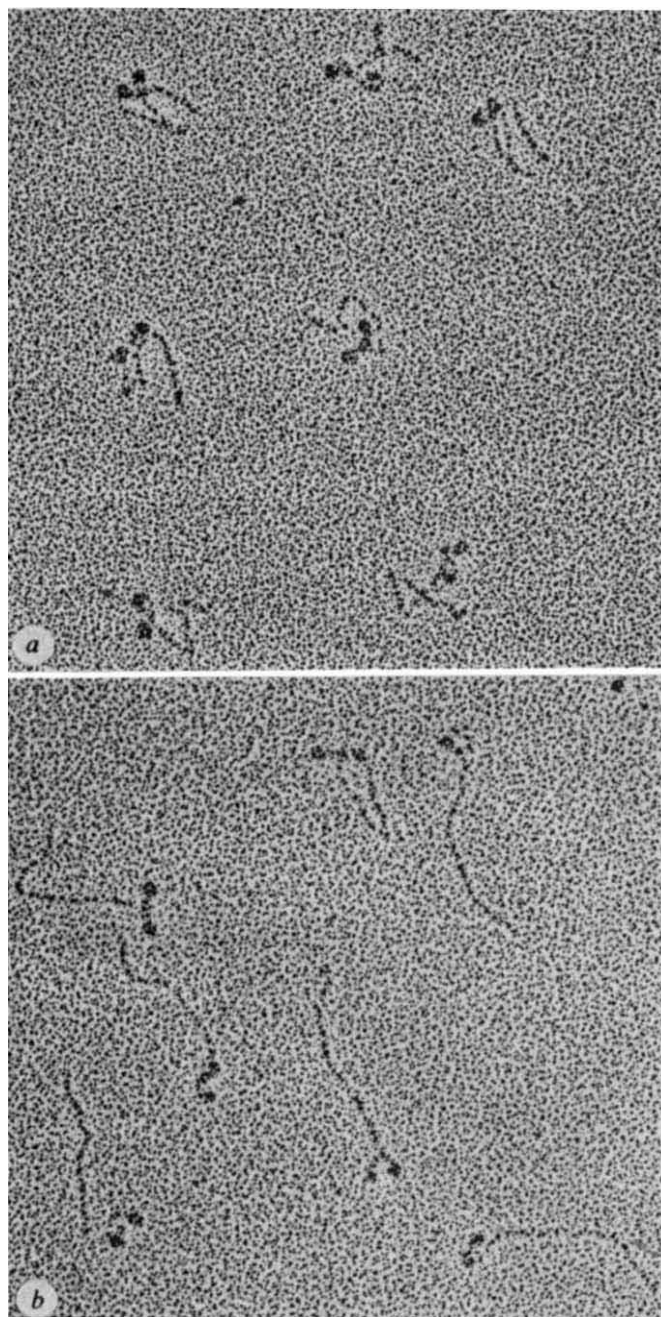


Fig. 1 Rotary-shadowed thymus myosin molecules: *a*, nonphosphorylated; *b*, during filament assembly, ~70% phosphorylation of 20 K- M_r light chains ($t = 23.5$ min, $A_{340} = 0.155$; see Fig. 3). For shadowing, 50- μ l aliquots of myosin (0.5 mg ml^{-1}) were removed from the assay solution in Fig. 3 when required and diluted into 450 μ l 0.15 M NaCl, 0.2 mM EGTA in 50% (v/v) glycerol. Specimens were sprayed from a glass atomizer on to freshly cleaved mica^{11,21-23}, rotary-shadowed with platinum at an angle of 5–6° and a pressure of 3×10^{-5} mbar, and then carbon-coated. Replicas were examined in a Philips EM400 electron microscope operated at 80 kV and micrographs were recorded at a magnification of 27,500. As molecules are sprayed in non-volatile salt, fine structure is rather inferior to that observed with volatile salt, for example, ammonium formate (unpublished results).

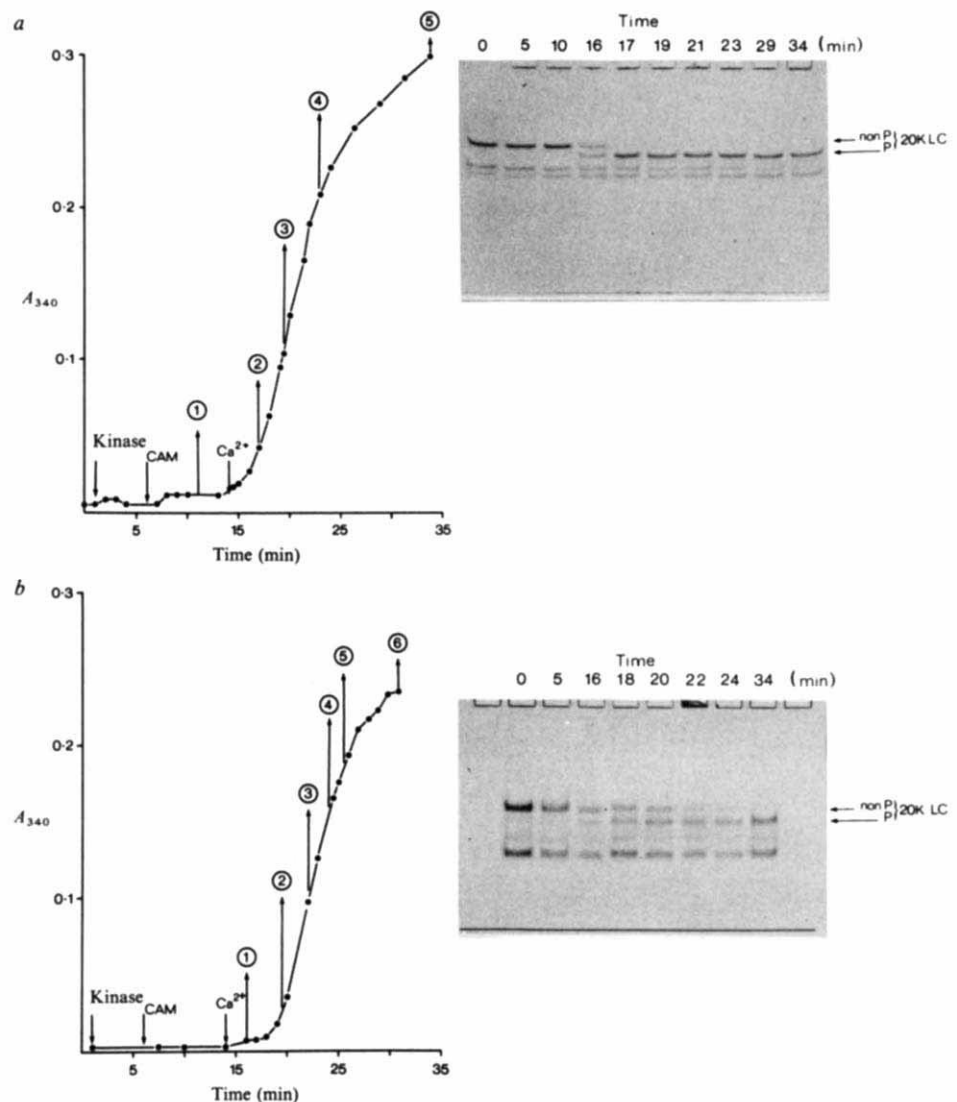
Fig. 2 Rotary-shadowed gizzard myosin molecules: *a*, nonphosphorylated; *b*, during filament assembly, ~90% phosphorylation of 20 K- M_r light chains ($t = 18$ min, $A_{340} = 0.060$; see Fig. 3). Shadowing as described in Fig. 1 legend.

dramatic change in the conformation of the folded myosin molecules; the tails unfold to produce extended molecules (Figs 1*b*, 2*b*, 4*b*) similar in appearance to those observed when the myosins are dissolved in 0.6 M salt. These extended molecules then assemble into filaments as shown by the increase in turbidity (Fig. 3) and verified by negative staining/electron microscopy. The correlation between light-chain phosphorylation and

unfolding of the tails is reasonably good for both thymus and gizzard myosins (see Fig. 3 and Table 1). If one examines light-chain phosphorylation and filament assembly as measured by the increase in turbidity, however, the myosins behave differently. Although the light chains in the gizzard myosin are rapidly phosphorylated there is a distinct lag before filament assembly occurs (see, for example, $t = 17$ min in Fig. 3) which allows us to observe the extended molecules before their incorporation into filaments. With the thymus myosin, however, filament formation more closely follows light-chain phosphorylation and thus at any given time in the assembly assay one sees fewer extended molecules (Table 1).

These results demonstrate that *in vitro* in physiological conditions of pH, ionic strength and ATP concentration, the non-phosphorylated form of vertebrate non-muscle and smooth muscle myosin is a monomer which cannot form filaments

Fig. 3 The regulation of gizzard (a) and thymus (b) myosin filament assembly is controlled by phosphorylation of the 20K- M_r light chains. Left, time course of filament assembly monitored by turbidity; right, time course of phosphorylation monitored by urea-glycerol gel electrophoresis. Nonphosphorylated gizzard or thymus myosin filaments (0.5 mg ml^{-1}) in 0.15 M NaCl , 0.2 mM EGTA , 10 mM Mg^{2+} , $25 \text{ mM imidazole pH } 7.0$, $0.25 \text{ mM dithiothreitol}$ were disassembled by incubating at 20°C with 2.5 mM MgATP for 5 min and were centrifuged at $100,000g$ for 30 min to remove any filament aggregates (at least 90% of the gizzard and 70% of the thymus filaments disassembled). The disassembled myosins were incubated at 20°C with $0.5 \mu\text{g ml}^{-1}$ gizzard muscle myosin light-chain kinase and the turbidity at $\lambda = 340 \text{ nm}$ was recorded to monitor filament assembly^{1,2,6}. To activate the kinase, $5 \mu\text{g ml}^{-1}$ thymus calmodulin (CAM) and 0.3 mM Ca^{2+} were added. Aliquots were taken at the numbered, circled points for rotary shadowing and electron microscopy and the numbers of folded and extended molecules in each sample were estimated (Table 1). The final level of turbidity reached was very similar to that of the initial filament solution before MgATP addition. Aliquots were also taken for negative staining to verify in the electron microscope that the changes in turbidity were in fact monitoring filament assembly⁵. The levels of phosphorylation of the 20K- M_r light chains of thymus and gizzard myosins during the assembly assays were analysed by urea-glycerol gel electrophoresis²⁴. Aliquots were removed from the thymus and gizzard myosin turbidity assays at the times shown above each gel. The samples were processed for electrophoresis by precipitation with a final concentration of 3% trichloroacetic acid (w/v) and the precipitated proteins centrifuged and washed with cold acetone. They were dissolved in 9 M urea , $25 \text{ mM Tris-122 mM glycine pH } 8.6$ buffer solution and run on 40% glycerol-10% polyacrylamide gels²⁴. The arrows indicate the positions of the nonphosphorylated (nonP) and phosphorylated (P) 20K- M_r light chain (20K LC).



apparently because of its folded shape. The key feature of the folded structure seems to be the binding of a portion of the myosin tail (generally near to the distal or second hinge) to the neck region of the myosin head(s) (Figs 1a, 2a, 4a) near the putative site of the regulatory light chains¹³⁻¹⁵. The folded state does not seem to be maintained by specific charge interactions between the different segments of the myosin tail (see ref. 16) as the segments are generally seen clearly separated from each other (Figs 1a, 2a, 4a).

When the regulatory light chains are phosphorylated, the myosin tail is released from the neck and unfolds to form the extended structure. In many of these extended molecules, especially those of the gizzard myosin, the distal hinge region is still frequently observed (Fig. 4b). The extended molecules are now able to assemble into filaments and the simultaneously activated (that is, phosphorylated) myosin heads are able to interact with actin to generate the force for movement¹⁷. Thus *in vitro*, at physiological ionic strength and pH, MgATP binding to nonphosphorylated thymus and gizzard myosin heads, presumably at the MgATPase active sites^{2,3}, produces a major change in the conformation of the whole myosin molecule and the covalent addition of a phosphate group to the 20K- M_r light chains is sufficient to reverse this change.

Do these *in vitro* observations have any relevance to the state of myosin assembly in living vertebrate non-muscle and smooth muscle cells? In non-muscle cells the folding of myosin into a compact form may facilitate its transport to the intracellular site where motile activity is required. Phosphorylation of the light chains might then induce the formation of filaments, which could act in combination with actin filaments to produce force. It is also possible that in truly *in vivo* conditions (for example, cytoplasmic viscosity and other interacting proteins) the changes in myosin conformation induced by phosphorylation may be smaller than we have observed, or may not occur at all. Myosin has recently been localized in non-muscle cells at the level of resolution of the electron microscope using immunocytochemical techniques^{18,19}, and thus its precise *in vivo* molecular organization at rest and during motile activity may soon be established in order to test the relevance of our observations. In vertebrate smooth muscle, electron microscopy of rapidly frozen tissue has demonstrated that thick filaments are present in the relaxed state even though the myosin contains nonphosphorylated light chains²⁰. The discrepancy between these ultrastructural observations and the *in vitro* results^{1-5,9,10} with isolated gizzard myosin is difficult to resolve at present and may be due to factors or conditions present in the muscle which

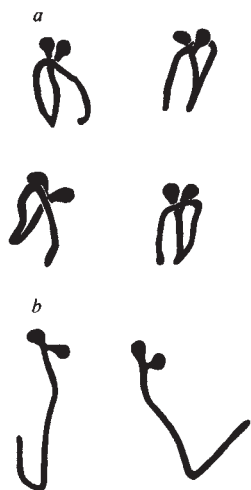


Fig. 4 Tracings of rotary-shadowed thymus and gizzard myosin molecules: *a*, nonphosphorylated, folded molecules. Most of the molecules we observed show myosin tail association with the myosin neck; Onishi and Wakabayashi¹⁰ found a similar association of the neck with the tail. They observe, in addition, that the first two segments of the tail (proximal to the heads) run in closer apposition to each other than we have seen, and that the heads are generally bent back towards the tail (which we did not observe). These differences may arise from differences in the salt conditions used for drying the molecules before shadowing. *b*, Phosphorylated unfolded molecules. $\times 160,000$

are absent from the *in vitro* studies. Nonetheless, it would be surprising if the phosphorylation-dependent changes we observe in smooth muscle myosin *in vitro* do not have some significance *in vivo*; for example, phosphorylation may change the flexibility of the myosin molecules within filaments *in vivo*. Changes in myosin flexibility might also occur in striated muscles in response to ATP binding and light-chain phosphorylation. In this case (and also possibly in smooth muscle) where the filaments do not disassemble, interaction could occur between the neck of one myosin molecule and the tail of another in the same filament (due to axial staggering of molecules) and the changes induced by ATP binding and phosphorylation could alter this interaction.

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Cloning of the initiation region of a mammalian chromosomal replicon

Nicholas H. Heintz, Jeffrey D. Milbrandt, Kay S. Greisen & Joyce L. Hamlin

Department of Biochemistry, University of Virginia School of Medicine, Jordan Medical Educational Building, Charlottesville, Virginia 22908, USA

In eukaryotic cells, each chromosome is divided into several thousand tandemly arranged replicons, each synthesized at a characteristic time during the S period^{1,2}. Although as yet no eukaryotic DNA sequence known for certain to be a eukaryotic chromosomal replication origin has been isolated, electron microscopic^{3,4} and biochemical^{5–7} evidence suggests that eukaryotic DNA synthesis is initiated at specific sites. We have attempted to establish a system in which functional chromosomal origins could be identified *in vivo* before their isolation by molecular cloning. For this purpose, we have developed a methotrexate-resistant Chinese hamster ovary cell line (CHOC 400) that contains 1,000 copies of a 135-kilobase (kb) early-replicating sequence⁸, and which includes the gene for dihydrofolate reductase (DHFR). We have shown that initiation of DNA synthesis within each repeated unit (amplicon) is restricted to a small subset of restriction fragments at the beginning of the S period⁹, suggesting that these fragments contain or flank origins of DNA synthesis. Here we report molecular cloning and restriction mapping experiments showing that all of these early-labelled fragments (ELFs) are derived from a single locus within each repeated unit. This result implies that synthesis of each amplicon initiates from a single origin of replication at the onset of S, and that an amplicon is formally equivalent to a replicon.

Figure 1, lane 1, shows the characteristic autoradiographic pattern of amplified restriction fragments from *EcoRI* digests of CHOC 400 genomic DNA labelled with ¹⁴C-thymidine during log phase growth. Of the 25–30 *EcoRI* restriction fragments that comprise the amplified DHFR domain, only a small subset is labelled with ¹⁴C-thymidine during the first 40 min of the S period (Fig. 1, lane 2). To characterize the ELFs further, and as a step towards the isolation of a biologically functional origin of DNA synthesis, we excised two prominent *EcoRI* ELFs at 11.6 and 6.1 kb (designated ELF-C and ELF-F) from a preparative agarose gel, and used these fragments separately as hybridization probes to screen a cosmid library constructed from CHOC 400 genomic DNA (see Fig. 2 and legend). Replica filters containing a total of ~11,000 recombinant colonies were screened with each probe individually in the presence of excess unlabelled CHO DNA. Small DNA preparations from 72 positive colonies were digested with *EcoRI*, the digests were electrophoresed along with fractions C and F, and were Southern blotted. These digests were then screened sequentially with each radioactive probe. On the basis of hybridization of each probe to *EcoRI* fragments of the correct size, three of these cosmids appeared to contain both ELF-C and ELF-F in their entirety. Of these three, S21 spanned the longest segment of genomic DNA (28 kb), and was therefore selected for restriction mapping. In Fig. 3, the fragments in CHOC 400 genomic DNA that hybridize to S21 are seen to include the two bands recognized by the original gel fractions C and F. In addition, comparison of the hybridization signals detected in CHO and CHOC 400 digests indicates that S21 is derived from the amplified domain of the CHOC 400 genome. Note also that both fractions C and F, and also S21 hybridize to an *EcoRI* fragment of ~8.8 kb in the digest of CHOC 400 DNA (arrow, Fig. 3). The band observed in Fig. 3 is more intense than usually observed (for example, see Fig. 1, lane 3, *EcoRI* digest), because

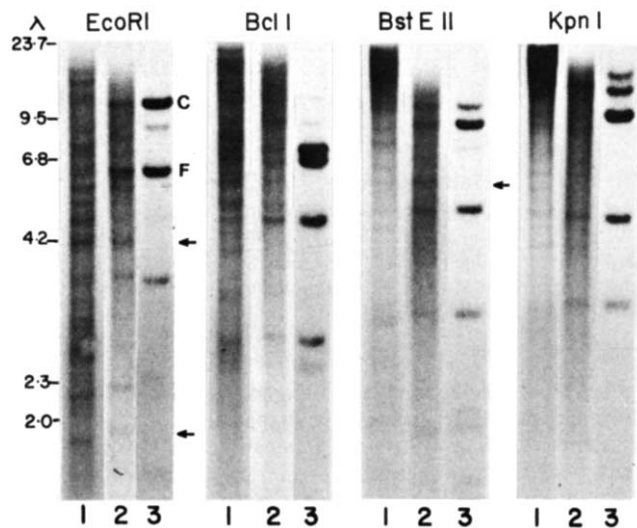


Fig. 1 Comparison of the restriction patterns of early-labelled CHOC 400 DNA to the corresponding hybridization patterns of clone S21. Lanes 1, the ^{14}C autoradiographic patterns of 3 μg of the indicated digest of CHOC 400 DNA labelled during log phase growth. Lanes 2, the ^{14}C autoradiographic pattern of 2.5 μg of CHOC 400 DNA labelled during the first 40 min of S. Lanes 3, the ^{32}P autoradiographic pattern obtained by hybridizing S21 to the digests depicted in lanes 1.

Methods: CHOC 400 cells were synchronized in early G_1 by starvation for isoleucine for 48 h. Cells were then collected at the G_1/S boundary by incubation with complete medium containing 5 $\mu\text{g ml}^{-1}$ aphidicolin. The aphidicolin was removed, and cultures were labelled for the first 40 min of S with 1.0 $\mu\text{Ci ml}^{-1}$ ^{14}C -thymidine medium (50–60 mCi ml^{-1} , Amersham). Control cultures were labelled for 16 h with 0.1 $\mu\text{Ci ml}^{-1}$ ^{14}C -thymidine medium. High molecular weight genomic DNA was prepared as described previously⁹, and was digested with *EcoRI*, *BclI*, *BstEII* and *KpnI*. The digests were electrophoresed on a 0.8% agarose gel and were Southern blotted. The *in vivo* labelling pattern of early-replicating DNA was obtained by exposing the blot to X-ray film for 14 days; exposure time for the log-labelled digests was 2 days. The log-labelled digests were then probed with clone S21 labelled with ^{32}P by nick-translation¹⁵, and the hybridization pattern was visualized by autoradiography through Saran Wrap with X-ray film for 2.0 days. The log-labelled samples migrated slightly faster than the corresponding early-labelled digests due to concentration differences in the samples. Hybridization conditions were as described in Fig. 2 legend. Arrows indicate ELF's not hybridized by S21. Size markers are the *HindIII* fragments of λ DNA.

this autoradiogram was overexposed so as to bring out the smaller hybridizing species. This band is not present in *EcoRI* digests of genomic DNA from other independently-derived methotrexate-resistant Chinese hamster cell lines, nor is this band present in S21 (not shown). As this fragment hybridizes to fraction C by itself (not shown), we believe that it represents a variant C fragment that is present in ~10% of the amplified DHFR domains in CHOC 400. The C and F fragments cloned in S21 were shown to be identical to the gel fractions used as probes on the cosmid library by comparing their respective hybridization patterns on Southern blots containing seven different restriction digests of CHOC 400 genomic DNA (hybridization data not shown). This result confirmed that S21 does indeed contain both ELF-C and ELF-F, two of the *EcoRI* fragments that are labelled in early S.

We next compared the restriction pattern of *in vivo* labelled ELF's with the restriction and hybridization patterns of S21. DNA labelled with ^{14}C -thymidine during the first 40 min of the S period was digested with several different restriction enzymes, and Southern blots of these digests were subjected to autoradiography (Fig. 1). As can be seen, a characteristic subset

Separate an *EcoRI* digest of CHOC 400 genomic DNA on a low melting agarose gel.

Excise two *EcoRI* ELF's (C and F) from the gel, and nick-translate each fraction with ^{32}P -dCTP.

Screen a CHOC 400 cosmid library (Sau3a partial digest cloned into the BamHI site of PHC79) with each probe separately.

Digest positive colonies with *EcoRI*, and probe digests with labelled C and F fractions.

Select clones that contain C and F in their entirety, nick-translate with ^{32}P -dCTP, and use as probes on digests of CHOC 400 and CHO DNA, to determine whether they arise from the amplicon.

Select a clone that contains both C and F that is also amplified (S21).

Hybridize ^{32}P -labelled S21 to CHOC 400 genomic digests. Compare pattern to ^{14}C -labelled ELF's.

Map S21 and corresponding region in CHOC 400 genomic DNA.

Fig. 2 Strategy for isolation of the initiation locus. CHOC 400 DNA (100 μg) was digested to completion with *EcoRI* and was electrophoresed on a preparative low-melting agarose gel (Bethesda Research Labs) in the presence of ethidium bromide until all fragments less than 4 kb had run off the gel. Ethidium bromide bands migrating at the positions corresponding to *EcoRI* ELF-C and *EcoRI* ELF-F were then excised from the gel, and the DNA from each fraction was recovered by phenol extraction of the melted agarose as described previously¹⁴. DNA from each fraction (1.0 μg) was labelled with ^{32}P by nick-translation¹⁵, and 2×10^5 c.p.m. ml^{-1} (10^8 d.p.m. per μg) of each probe was hybridized to replica Southern blots¹⁶ of *EcoRI* digests of early-labelled CHOC 400 DNA for 16 h at 42°C using dextran sulphate, as described previously¹⁷. Sonicated CHO genomic DNA (1.0 $\mu\text{g ml}^{-1}$) was included in the hybridization solutions to reduce background due to highly repetitive DNA. Blots were washed at 55°C in 1×SSC, 0.1% SDS for 30 min before autoradiography. Comparison of the ^{14}C -thymidine autoradiographic pattern with the corresponding ^{32}P autoradiographic pattern confirmed that the gel fractions contained fragments C and F. Each probe was then used to screen sets of replica filters containing a total of 1,000 recombinant cosmid colonies constructed from CHOC 400 genomic DNA (J.D.M. *et al.*, in preparation). Filters were hybridized in 2×SSC, 2×Denhardt's, 0.1% Triton X-100, 100 $\mu\text{g ml}^{-1}$ sonicated herring sperm DNA, 1 $\mu\text{g ml}^{-1}$ CHO DNA for 12 h at 55°C, and were washed in 1×SSC, 0.1% SDS at 45°C before autoradiography. Small preparations of cosmids from each of the 72 colonies that yielded the darkest signals were then digested with *EcoRI*, and the digests were electrophoresed along with the original gel fractions C and F. These digests were then Southern blotted¹⁶, and each blot was probed with ^{32}P -labelled gel fractions C and F sequentially. Large cosmid preparations from colonies that contained sequences homologous to C, to F or to both C and F were nick-translated with ^{32}P , and were then used to probe replica blots of *EcoRI* digests of CHOC 400 and CHO DNA to confirm their origin from the amplified domain. Hybridization conditions were as described previously¹⁷. Cosmid S21, which spanned the longest distance of amplified genomic DNA, was selected for further study.

of restriction fragments is labelled in each digest (Fig. 1, lanes 2), and these ELF's represent a small portion of the total number of amplified bands labelled in corresponding digests of DNA from log phase CHOC 400 cells (Fig. 1, lanes 1). The synthesis of these ELF's is restricted to the early S period, as they are not labelled at later times in S when different subsets of the amplified fragments are replicated⁹. When these same blots are subsequently probed with ^{32}P -labelled S21, virtually all of the ELF's are recognized, and are therefore derived from a single

Fig. 3 The genomic insert of S21 is derived from the amplified domain. 32 P-labelled gel fractions C and F and cosmid S21 were hybridized to replica Southern blots of *Eco*RI digests of CHO and CHO 400 DNA. After washing, the blots were exposed to X-ray film for 2 days. 400 indicates CHO 400 genomic digests; CHO indicates DNA samples from the methotrexate-sensitive parental CHO cell line. Sizes of the genomic *Eco*RI fragments (including C and F) recognized by cosmid S21 are shown, and a cross-hybridizing species at 8.8 kb is indicated with an arrow.

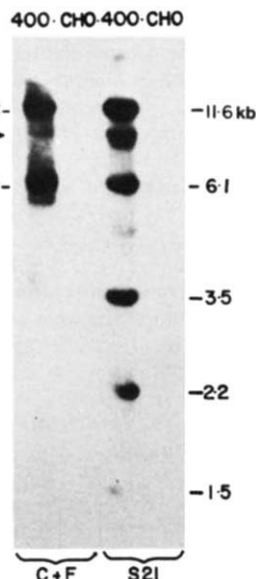
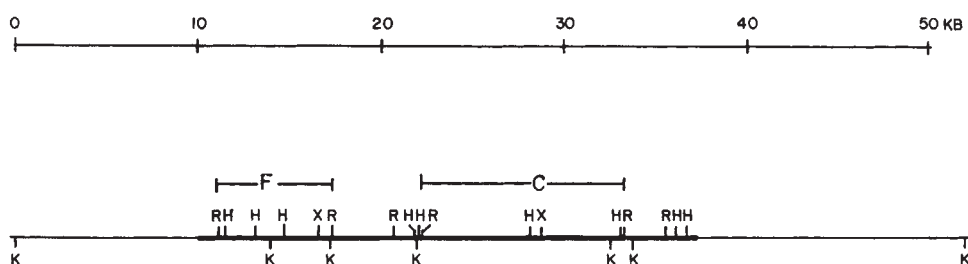


Fig. 4 Restriction map of the initiation region of the amplified DHFR domain. The recombinant cosmid S21 was mapped by electrophoretic analysis of the appropriate combinations of restriction digests of the indicated enzymes, and the map of the clone was compared with the corresponding region in the genome of CHO 400 by hybridization experiments. Sites presented are those in the genome.

The bold line delineates the genomic sequences contained in cosmid S21. *Eco*RI fragments corresponding to the original ELF's C and F are indicated. Abbreviations used: H, *Hind*III; K, *Kpn*I; R, *Eco*RI; X, *Xho*I.



genomic locus. For instance, *Kpn*I yields four distinct ELF's at 15, 10.6, 4.8 and 3.25 kb. All of these fragments are recognized by S21. A fifth band at 18 kb is recognized by cosmid S21, but is only weakly labelled during the first 40 min of S. *Eco*RI yields seven prominent ELF's at 11.6, 6.1, 4.2, 3.5, 2.2, 1.75 and 1.54 kb. Of these, all but the 1.75- and 4.2-kb bands (arrows) are represented in S21. Mapping of overlapping cosmids indicates that these fragments are derived from sequences flanking S21 (not shown). *Bst*EII yields ELF's at 13.1, 10.4, 6.3, 5.1, 3.15 and 1.8 kb, and hybridization with S21 shows that all of these, with the exception of the 6.3-kb fragment (arrow), are recognized by this clone. *Bcl*I yields ELF's at 11.0, 8.8, 6.8, 6.4, 4.3, 2.5 and 2.1 kb, all of which are hybridized by S21. Similar results are obtained with other enzymes, and we have consistently found that ELF's not represented in S21 are present in other overlapping cosmids containing flanking sequences (not shown).

To confirm that S21 represents a non-rearranged DNA segment equivalent to that found in the CHO 400 genome, S21 was mapped with restriction enzymes (Fig. 4), and the map of the clone was compared with that of genomic DNA by Southern blot analysis (hybridization data not shown). The results of these experiments demonstrate that S21 is indeed derived from the amplified DHFR domain in CHO 400 cells, and that its organization is identical to that of the initiation region as it exists in the cell. Comparison of the restriction map of S21 with the autoradiographic patterns of CHO 400 DNA labelled *in vivo* suggests that the most intense labelling occurs near the *Eco*RI site defining the right-hand end of fragment F (Fig. 4). Other fragments derived from this region, such as the 15-kb *Kpn*I fragment overlapping the left-hand end of S21, also seem

to be the most heavily labelled in early S. In contrast, fragments distal to this region, such as the 18-kb genomic *Kpn*I fragment which overlaps the right-hand end of S21, are only weakly labelled during the same pulse period.

Thus, we have shown by molecular cloning and restriction mapping that initiation of replication of an amplified chromosomal sequence occurs from a single locus, and therefore consider it likely that cosmid S21 contains a functionally defined origin of DNA replication. If initiation of replication occurs from only a single site within each amplified DHFR domain, then the unit repeated sequence, by definition, is equivalent to a chromosomal replicon. This suggests a model in which gene amplification is the result of the repeated synthesis of a single replicon in one S period. This model is suggested by several experimental observations. The size of the amplified domain (135 kb) is well within the range predicted for replicons by fibre autoradiographic studies¹⁰. This size also approximates that published for supercoiled DNA loops that have been implicated as the unit of replication in mammalian cells¹¹⁻¹³.

The cloning of the initiation region of the amplified DHFR domain should allow us to isolate a *bona fide* chromosomal origin of DNA replication. As we have also cloned a large part

of the remainder of the amplified region, we hope to be able to study the replication of this entire domain and its relationship to the amplification process itself.

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Assembly of two types of tubules with putative cytolytic function by cloned natural killer cells

Eckhard R. Podack

Department of Immunology, Research Institute of Scripps Clinic, La Jolla, California 92037, USA

Gunther Dennert

Autoimmune and Neoplastic Disease Laboratory, The Salk Institute for Biological Studies, San Diego, California 92138, USA

The formation of ultrastructural membrane lesions of varying size during cell mediated cytotoxicity effected by human peripheral blood leukocytes was recently reported by Dourmashkin *et al.*¹ and Henkart *et al.*². Using cloned mouse natural killer (NK) cells³ as effectors and YAC-1 cells or rabbit erythrocytes as targets, we now report two types of membrane lesions with inner diameters of 16 ± 2 nm and ~ 5 nm, respectively. These lesions arise by membrane insertion of tubular complexes that may be assembled from subunits during the cytolytic reaction.

The tubules are detected on target membranes by immune electron microscopy and appear to form transmembrane channels as seen in ultrathin sections. Both tubules are partially purified by membrane extraction with SDS and gel filtration in deoxycholate containing buffer. Based on the correlation of tubule assembly and cytotoxicity and on their detection on target membranes, we suggest that both types of tubules may be related to cytotoxicity.

NK effector cells and targets, YAC-1 cells or rabbit erythrocytes + concanavalin A (Con A), were incubated for 4–16 h, membranes were prepared by differential centrifugation and used for negative staining electron microscopy. Figure 1a, c shows the ultrastructure of the two types of membranes lesions. Detergent extraction of membranes as reported below indicates that the lesions are formed by membrane bound, tubular complexes. The larger tubule, type 1 (T1), has an inner and outer diameter of 16 ± 2 nm and 26 ± 2 nm, respectively. Figure 1b shows for comparison human and mouse (inset) complement lesions. Substructure (arrow, Fig. 1a) within T1, formation of incomplete rings, double rings (large arrowheads) and of irregular structures (Fig. 1d) suggests that the structure of T1 may be polymeric. On vesicles T1 structures project about 12 nm above the membrane surface (parallel arrowheads in Fig. 1e, f). The end of T1 opposite to its membrane binding site bears a ~ 3 nm thick torus of ~ 26 nm outer diameter that tends to interact by top-to-top aggregation (Fig. 1f). No circular

Fig. 1 Comparison of the NK-cell lesions (T1 and T2) with human and mouse complement lesions. *a*, T1 formed by incubation of NK cells with rabbit erythrocytes in the presence of $20 \mu\text{g ml}^{-1}$ Con A (arrows). Incomplete rings and double rings are indicated by arrowheads. *b*, Human complement lesions and in the inset a mouse complement lesion on rabbit erythrocyte membranes. The putative C5b-8 attachment is indicated by arrows. *c* Shows the T1 and T2 (large arrowheads) on membrane fragments formed during incubation of NK cells with YAC-1 target cells. *d* Shows irregular T1 complexes (arrows). *e* and *f* show T1 complexes bound to vesicles. The parallel arrowheads show the 12 nm projection of the tubule above the membrane and the torus. *g*, A rabbit erythrocyte membrane is labelled with colloidal gold coupled to goat anti-rabbit erythrocyte IgG. The arrowheads depict gold particles and the arrows T1 complexes on the same membrane fragment. Scale bar, 57 nm in all panels. Experimental conditions: Cloned mouse NK cells were grown in continuous culture as described previously³. Effector target ratios were 1:1. When rabbit erythrocytes were used as targets, $20 \mu\text{g ml}^{-1}$ Con A was added. No Con A was added when YAC-1 cells were used as targets. Before incubation all cells were washed three times with serum-free culture medium containing 0.5% purified human serum albumin and the antibiotics penicillin, streptomycin, gentamycin and Fungizone. Cell mixtures containing 10^6 effector cells per ml were centrifuged (400 r.p.m.) and incubated for 16 h at 37°C in all panels shown (except panel 2). To obtain membrane fragments, the cell suspension was vortexed for 1 min and then incubated for 1 h at 37°C with $100 \mu\text{g ml}^{-1}$ trypsin to release fragments from intact cells and vortexed again. Intact cells, nuclei and other debris were separated by 5 min centrifugation at 1,000 r.p.m. and the pellet was discarded. The supernatant was centrifuged for 15 min at 35,000g. The membranes were resuspended in $200 \mu\text{l}$ Tris-Cl buffered saline, pH 7.2, containing 0.02% NaN_3 and incubated for 16 h at 20°C with $100 \mu\text{g ml}^{-1}$ trypsin and chymotrypsin and washed twice with water. Lesions were also detectable without digestion albeit at lower contrast. Human complement lesions were assembled on rabbit erythrocytes as described previously⁷. All membrane preparations were negatively stained with uranyl formate and then imaged in a Hitachi 12A microscope at 75 kV accelerating voltage and 35,000-fold direct magnification. Affinity purified goat anti-rabbit erythrocyte IgG was labelled with 30–60 Å diameter colloidal gold⁴ and $5 \mu\text{g}$ gold tagged IgG was added to the trypsin, chymotrypsin treated membrane preparation. After incubation for 1 h at 37°C membranes were washed twice in Tris-buffered saline in the microfuge to remove unbound gold-IgG.

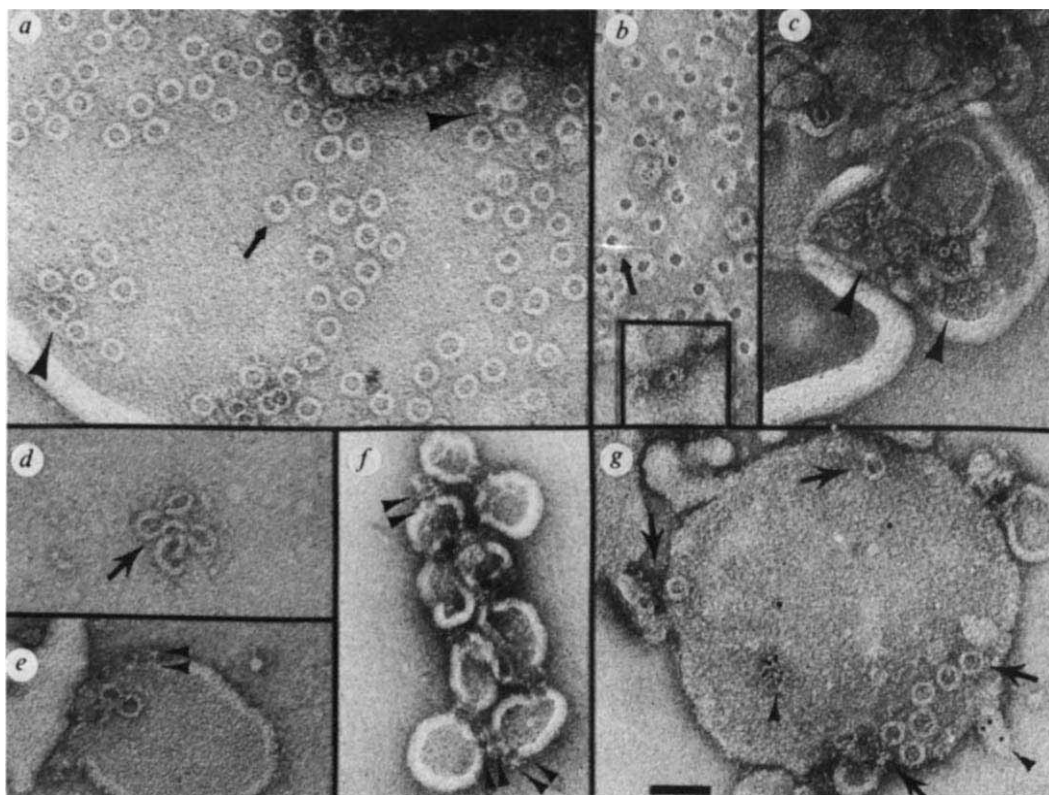
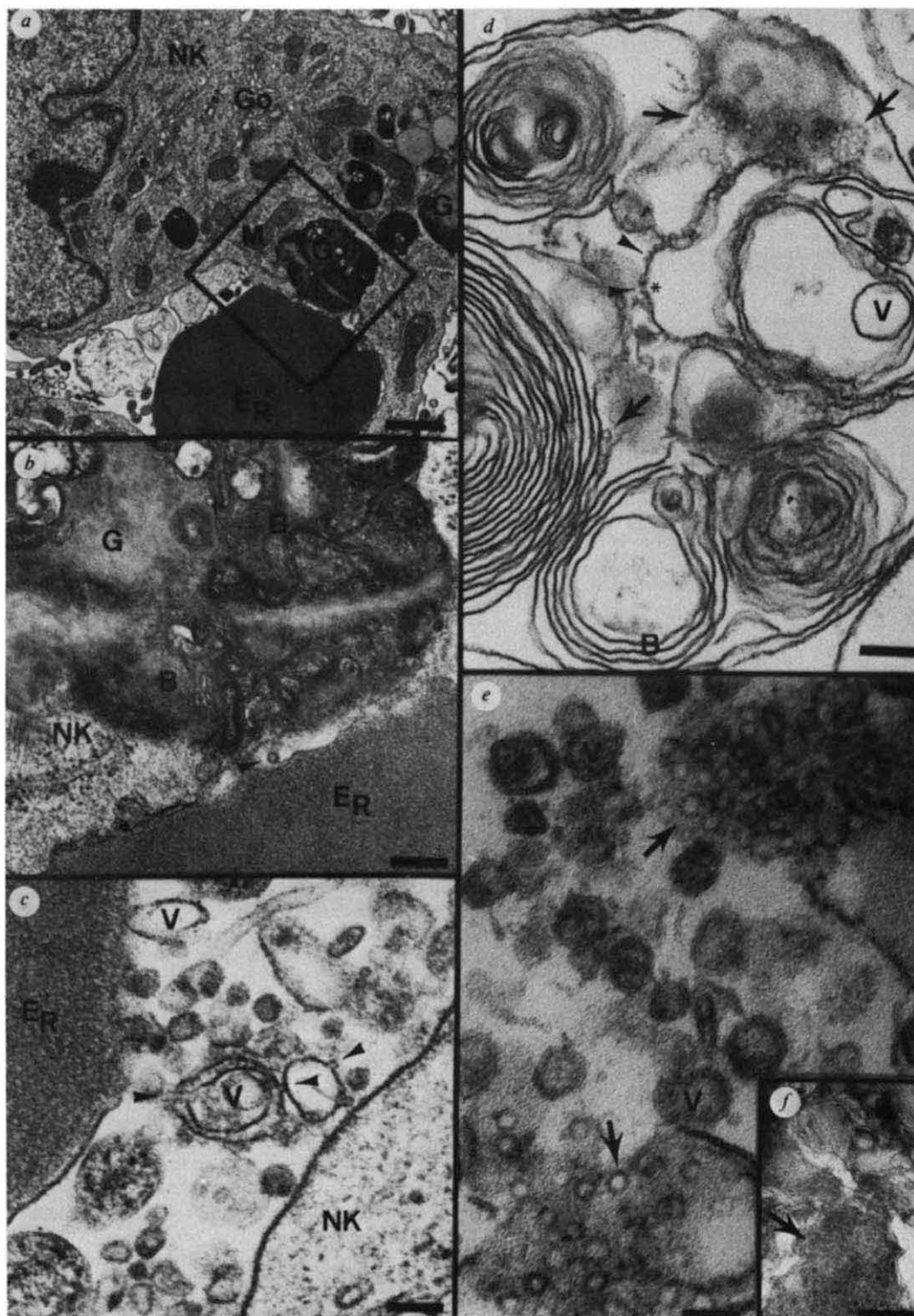


Fig. 2 Ultrastructural events revealed by thin sectioning of NK cells lysing rabbit erythrocytes in the presence of $20 \mu\text{g ml}^{-1}$ Con A. *a-c*, Samples were fixed after 4 h incubation. *d, e*, Samples fixed after 16 h with 1.5% glutaraldehyde, 2% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.2. All samples were post-fixed with osmium tetroxide and, after Epon embedding and ultrathin sectioning, positively stained with lead citrate, uranyl acetate. *f* Shows for comparison a negatively stained sample after 16 h incubation. Scale bars: *a*, 1.11 μm ; *b*, 270 nm; *c, d*, 111 nm; *e*, 55.5 nm; *f*, 114 nm. *a*, NK conjugate. N, nucleus; M, mitochondria; G, granules; Go, Golgi apparatus, of the NK cell (NK). *b*, Higher magnification of the granule (G) boxed in *a*. B, Multibilayer material. *c*, Another NK-erythrocyte conjugation area at higher magnification with vesicles (V) in the interstitial space. Axially sectioned T1 complexes are depicted with arrowheads. *d, e*, High power areas of extracellular material located close to NK cells after 16 h incubation and complete target lysis. Ring structures (arrows) and tubules (arrowheads) correspond to cross- or axially-sectioned T1s, respectively. The axially-sectioned tubule in *d* (*) has a length of 15.5 nm and an internal diameter of ~ 15 nm and forms a ~ 15 nm wide transmembrane channel. *f*, Extracellular membrane material obtained as described in Fig. 1 legend and negatively stained without fixation. Experimental details as given in legend to Fig. 1.



structures are detectable on incubation of rabbit erythrocytes or YAC-1 cells without NK cells for 16 h. In contrast, membrane association of tubules type 1 (T1) and type 2 (T2) can be seen when NK cells are incubated without targets in the presence of Con A; conditions in which autokilling of NK cells is observed. This finding shows that T1 and T2 are NK-cell products. Next it was shown that transfer of T1 to target rabbit erythrocyte membranes occurs (Fig. 1g). This shows an IgG-gold⁴ labelled target erythrocyte membrane fragment that also bears T1 complexes (arrows). The 3–6 nm IgG-gold particles (arrowheads) are clearly distinguishable as black dots from the negative stain. In control experiments, no detectable gold tagging of membrane fragments was found when rabbit eryth-

rocytes were omitted from the reaction mixture or when irrelevant gold-labelled IgG was used.

In time course experiments, NK-cell-mediated target lysis correlated with the number of membrane lesions detectable by electron microscopy. After 4 h incubation of NK cells with ^{51}Cr -labelled YAC-1 cells at a 1:1 ratio, specific ^{51}Cr release was 31%. Thirty-three per cent of the membrane fragments recovered bore two or more lesions. After 16 h incubation, 92% ^{51}Cr release correlated with 74% of all membranes bearing membrane lesions. Although it is not clear in this type of analysis whether the isolated membranes are representative of all membranes, the increase of membrane lesions with the time of incubation suggests a causal relationship to the lytic event. Both

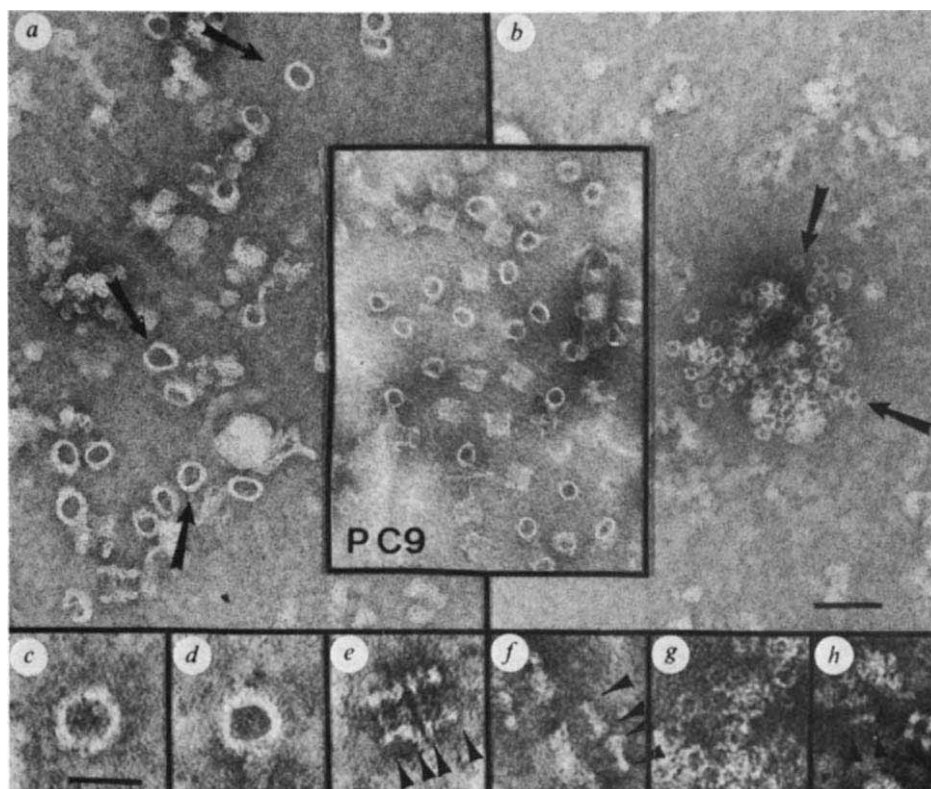


Fig. 3 Ultrastructure of the partially purified T1, T2 in comparison with human poly C9. The lower panels show higher magnifications of selected images of T1 (c-f) and T2 (g, h). T1 is shown in top views (arrows in a, c, d) and in side views (e, f arrowheads). Top-to-top association is seen in panels e, f. The T2 (b, arrows) has a tendency to side-by-side aggregation. The inset shows the ultrastructure of human poly C9 at the same magnification. Scale bars in a and b and in the inset correspond to 57 nm, in c-h to 28.5 nm. Partial purification of T1 and T2: Membrane fragments from 5×10^7 ^{35}S methionine-labelled NK cells and 5×10^7 YAC-1 cells were prepared without proteolysis as described in Fig. 1 legend. The pellet was then dissolved in 100 μl 2% SDS and boiled for 2 min. After addition of 300 μl 10% DOC dissolved in 20 mM Tris acetate, 0.09 M NaCl, 0.02 mM EDTA 0.02% NaN_2 , pH 8.7, the sample was applied together with a trace ^{125}I -IgM to a 0.5×20 cm column Sepharose CL-4B equilibrated with 1% DOC in the same buffer. Gel filtration proceeded at ~ 2 ml per h, 5-drop fractions being collected. ^{35}S -methionine-containing fractions eluting before the IgM peak were pooled, concentrated by ultrafiltration and used for electron microscopy.

lesion formation and lysis was inhibited by the carboxylic ionophore monensin⁵. Monensin, $1 \mu\text{g ml}^{-1}$ caused 70% inhibition of cytolysis which correlated with a 66% reduction of lesion formation after 16 h. EDTA caused killer cell disintegration and therefore its effects could not be assessed. The formation of membrane lesions always required the presence of highly cytolytic effector cells. Tumour cell lines or cell lines with low or no cytolytic activity as well as fresh lymphocyte preparations incubated with YAC-1 target cells in the absence or presence of Con A for up to 16 h did not give rise to lesions. Hence the lack of cytotoxicity always correlated with the lack of membrane lesion formation.

The T2 complex is much smaller than T1. It has an inner and outer diameter of ~ 5 nm and 10–12 nm, respectively (Fig. 1c, large arrowheads). We were not able to correlate the number of T2 complexes with lysis because of their small size. Both T1 and T2 are clearly distinguishable by size from human, mouse and bovine (lesions not shown) complement lesions (Fig. 1b, inset).

NK-cell-mediated rabbit erythrocyte lysis was also analysed in ultrathin sections. Figure 2a shows the morphology of a typical NK-target conjugate. The NK cell is seen as a large lymphocyte containing numerous dense granules⁵⁻⁷ that are in close contact to the target cell. Granules and the Golgi apparatus had previously been suggested to be involved in the cytolytic reaction^{2,5}. In Fig. 2b the granule which is in close contact with the effector target binding site contains vacuoles and numerous multilayer structures (B in Fig. 2b, d). Multilayer structures and vesicles are also detected in the interstitial space and in association with the rabbit erythrocyte (E_R) membrane, suggesting that they may arise from NK granules (Fig. 2c, d). After 4 h incubation (left panels) the targets are still recognizable by their haemoglobin content whereas after 16 h (right panels) they are all lysed. At high magnification (Fig. 2c-e), T1 is clearly seen in the extracellular space in association with vesicles and with multilayers. When sectioned perpendicular to its tubular axis, T1 appears as ring structure (arrows in all panels) with the same dimension as in the negatively stained samples. Sectioned parallel to its tubular axis T1 appears as ~ 160 Å long tubule (arrowheads in all panels). One of the axially sectioned T1 (Fig. 2d) appears to form a ~ 15 nm wide

transmembrane channel (*) at its binding site; others do not show a clear transmembrane channel possibly because the tubules are not sectioned centrally. The close association of ring structures and multilayers is also seen by negative staining (Fig. 2f). The T2, due to its small size, is not resolved by thin sectioning electron microscopy.

Because it is known that circular poly C9 is resistant to dissociation by SDS⁸, we tested whether T1 and T2 had similar properties. NK cells were metabolically labelled with ^{35}S -methionine and incubated with YAC-1 target cells. Cell membranes were solubilized in SDS and deoxycholate and gel-filtered on Sepharose CL4B. The high molecular weight peak of ^{35}S -methionine eluting before the IgM marker ($MW > 900,000$) was pooled and concentrated. Figure 3 shows electron micrographs of this preparation. In addition to extraneous material, typical large ring structures of T1 (Fig. 3a, c-f) and small rings of T2 (Fig. 3b, g, h) are clearly visible. The dimensions of top views correspond to those of the membrane lesions. In side views the length of T1 in solution is ~ 16 nm whereas it projects only ~ 12 nm above the surface when bound to membranes, suggesting a ~ 4 nm deep insertion. Top-to-top association of T1 is seen even after detergent extraction (Fig. 3e, f). The length of T2 in side views is ~ 11 nm. The inset in Fig. 3 shows SDS treated human poly C9 for comparison.

The studies reported here provide the first direct evidence that T1 and T2 are NK-cell products that may have the potential to form transmembrane channels as seen on axial sections through the centre of T1 and that they may be transferred to target cell membranes. As there is a strong correlation between target cell lysis and the number of lesions on membranes isolated from effector target cell mixtures, we suggest that both T1 and T2 participate in target cell lysis. The ~ 4 nm depth of membrane insertion would be sufficient for T1 to function as transmembrane channel. Based on the ultrastructural images, it appears possible that T1 may arise by circular polymerization of monomeric precursors. Thus, the formation of NK-cell lesions may be analogous to complement where it was shown that component C9, on circular polymerization, forms tubules that, when bound to membranes, appear as membrane lesions⁹⁻¹¹. The origin and insertional mechanism of T1 and T2 into target membrane remain speculative although circum-

stantial evidence suggests a role for the dense granules of NK cells and transfer from effector to target cells via membrane vesicles, in agreement with previously suggested secretory⁵ and fusion mechanisms².

One interesting aspect of these studies is the seemingly analogous molecular mechanism of membrane lesion formation by bacterial toxins (for example see refs 12, 13) by complement and by NK cells, suggesting a general biochemical mechanism. Whether complement proteins may be involved in cell-mediated cytotoxicity as has been discussed elsewhere⁴⁻¹⁷, or whether humoral and cellular cytotoxic proteins may have arisen by gene duplication as has been suggested¹⁸, or are completely unrelated, remains to be seen. The characterization of T1 which may be related to recently reported membrane lesions^{1,2} and of T2 which has not been detected previously may provide the key to the understanding of the molecular mechanism of cell-mediated cytotoxicity.

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A common region for proviral DNA integration in MoMuLV-induced rat thymic lymphomas

Philip N. Tschlis, P. Gunter Strauss & Li Fu Hu

Laboratory of Tumor Virus Genetics, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205, USA

Similarly to other mammalian and avian retroviruses that lack a transforming gene¹, moloney murine leukaemia virus (MoMuLV) causes no morphological transformation in infected tissue culture cells. However, following injection in an appropriate animal host, MoMuLV induces mainly thymic lymphomas after a long latency period^{2,3}. A common characteristic of neoplasms induced by retroviruses lacking transforming genes is their clonal origin⁴⁻⁹. Here we have generated MoMuLV-induced rat thymic lymphomas and confirmed their clonal nature. Furthermore, we took advantage of the clonality of these tumours to investigate the specificity of provirus integration in the tumour DNA. We reasoned that if several independently derived thymic lymphomas would contain the provirus integrated in the same region of cellular DNA, this would be a strong indication that this integration event is a contributing factor in oncogenesis. The results indicate that there is indeed a cellular DNA region (termed the *MLVI-1* locus) that serves as the substrate for proviral DNA integration in 5 out of 16 tumours we examined.

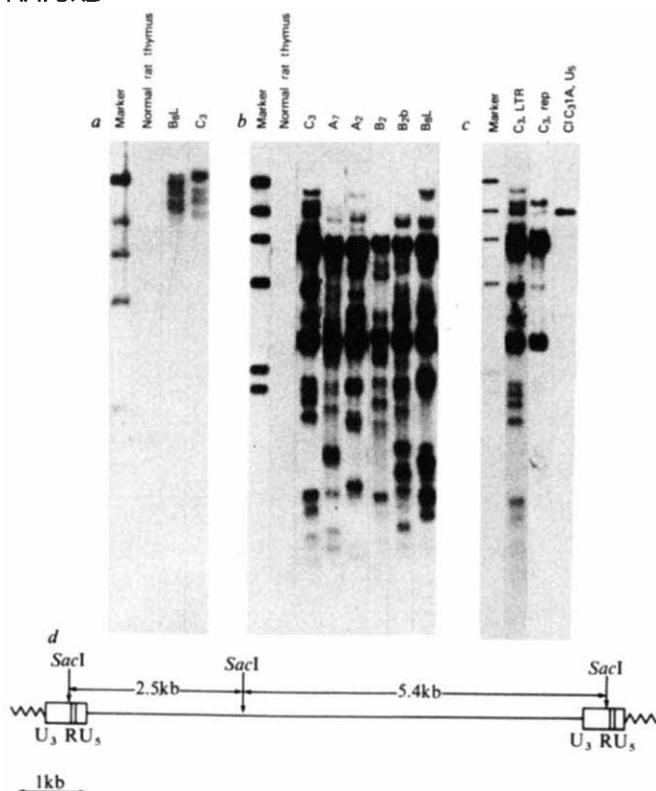


Fig. 1 *a, b*, Southern blotting³² of restriction endonuclease-digested normal rat thymus and tumour cell DNA hybridized to MoMuLV provirus DNA probes. *c*, Identification and characterization of provirus-host junction DNA fragments in C3 tumour cell DNA. *d*, Digestion of MoMuLV proviral DNA with *SacI* generates a 2.5-kb and a 5.4-kb DNA fragment^{22,23}. The marker in *a, b* and *c* is λ DNA digested with *HindIII*. This generates the following size fragments: 23.5, 9.7, 6.6, 4.3, 2.2 and 2.1 kb.

Methods: For *a*, 20 μ g of cellular DNA from each tumour and normal rat thymus was digested with *EcoRI* which does not cleave the DNA of the MoMuLV provirus²². The digested DNA was electrophoresed in 0.8% agarose gels and was transferred to nitrocellulose filters. Hybridization was performed using a nick-translated complete MoMuLV provirus DNA probe to detect all the virus-related sequences in tumour cell DNA. Represented here are one sample of normal thymus and two tumour DNAs. The normal rat thymus DNA which appears free of virus-related sequences in this exposure, shows two *EcoRI* fragments at 6 and 4 kb in heavily exposed films. For *b*, 20 μ g of cellular DNA from each tumour and normal rat thymus was digested with *SacI* (*d*). The digested DNA was electrophoresed in 0.8% agarose gels, transferred to nitrocellulose filters and hybridized to a nick-translated MoMuLV LTR DNA probe. We present here a representative sample of normal thymus and six tumour DNAs. The normal rat thymus is free of sequences hybridizing to the LTR probe. The two heavily hybridizing fragments at 5.4 and 2.5 kb represent the internal MoMuLV sequences shown in *d*. For *c*, 20 μ g of cellular DNA was digested with *SacI*, electrophoresed in 0.8% agarose gels, transferred to nitrocellulose filters and hybridized to the MoMuLV LTR or to the MoMuLV rep probe shown in Fig. 2*b*. Junction fragments as well as fragments derived from highly deleted proviruses containing only LTR sequences will hybridize only to the LTR probe while internal proviral DNA fragments will hybridize to both the LTR and the rep probe. The *SacI*-digested tumour cell DNA was fractionated by agarose gel electrophoresis into aliquots, each containing an individual provirus-cell junction DNA fragment. The cellular DNA of each aliquot was individually ligated into the *SacI* site of the bacteriophage λ vector λ gtWES.AB (ref. 21), and was packaged *in vitro* into phage particles³³. Phage plaques were screened using an LTR probe. Fortunately, the first clone we obtained (λ CIC₃1A) was derived from the cellular DNA aliquot containing the 9.5-kb junction fragment. The insert DNA of this clone is shown here hybridized to the U₅ probe shown in Fig. 2*b*. Other proviral DNA probes including the MoMuLV representative probe and a U₃ probe extending from the *SacI* to the *XbaI* site of the LTR did not hybridize to the CIC₃1A DNA.

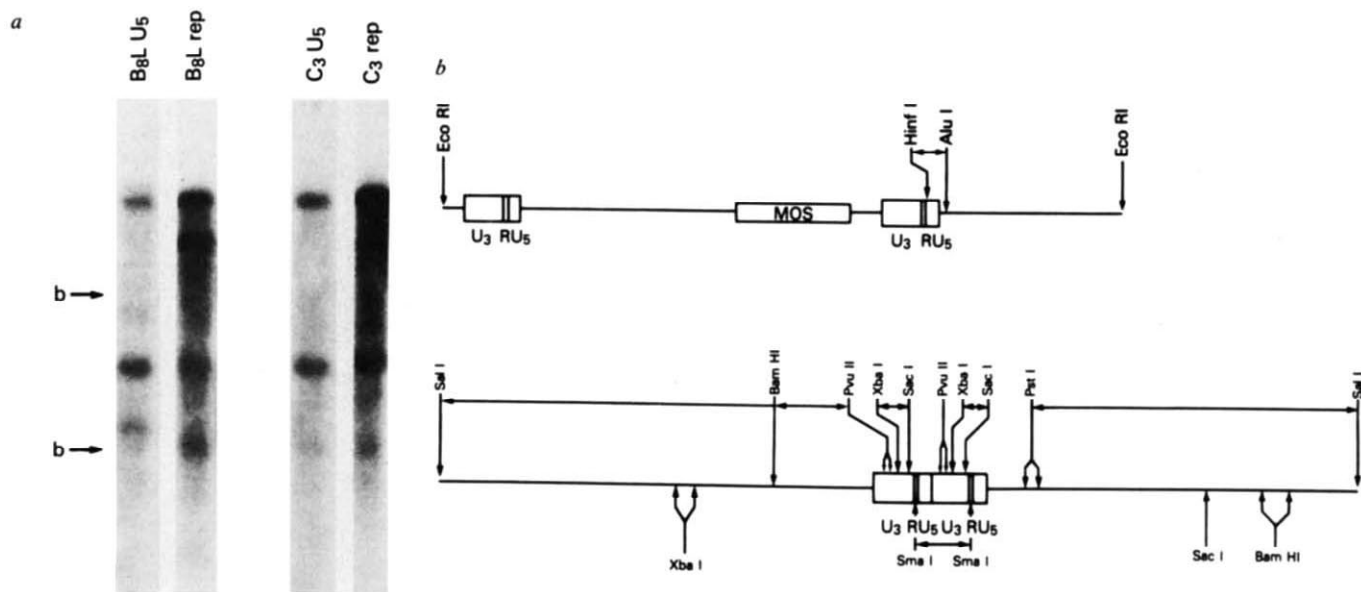


Fig. 2 Hybridization of tumour cell RNA to a U₅ and to a representative (rep) MoMuLV nick-translated DNA probe. *a*, Glyoxal-treated poly(A)-selected RNA³⁴ from tumours B8L and C₃ was electrophoresed in a 1% agarose gel, blotted onto diazobenzoyloxymethyl (DBM) paper³⁵ and hybridized to the U₅ or the rep MoMuLV proviral DNA probe. Note in tumour B8L a 2.4-kb mRNA transcript that hybridizes only to the U₅ probe and a 7-kb transcript that hybridizes only to the rep probe. Both transcripts are absent from tumour C₃. The arrows at 5.2 and 2 kb indicate the level of migration of marker 28S and 18S ribosomal RNA. *b*, Origin of proviral DNA probes. U₅ was derived from the m₁ clone of MoMSV³⁶, by digestion with *Hinf*I and *Alu*I. The rep MoMuLV probe is an equimolar mixture of the following fragments derived from the MoMuLV-cloned DNA: *Sal*I-*Bam*HI, *Bam*HI-*Pvu*II and *Pst*I-*Sal*I. This probe contains only 120 base pairs of U₃ sequences²⁴. The LTR probe is the *Sma*I-*Sma*I fragment derived from the MoMuLV-cloned DNA and subcloned with *Eco*RI linkers into pBR322. The U₃-specific probe contains the sequences from *Xba*I to *Sac*I.

The MoMuLV stock used in this study was derived by transfection of NIH 3T3 cells with molecularly cloned MoMuLV provirus DNA (provided by Dr Cha Mer Wei). Newborn Osborn Mendel rats were inoculated intraperitoneally with 50,000 plaque-forming units of MoMuLV and observed for signs of disease. Of 25 animals inoculated, 19 developed thymic lymphoma with a mean latency period of 5 months, and a single animal developed a mesenteric lymphoma after 8 months. The data presented here are the results of experiments studying the thymic lymphomas.

Our initial experiments investigated the clonality of these MoMuLV-induced rat thymic lymphomas by hybridization of MoMuLV proviral DNA probes to *Eco*RI- or *Sac*I-digested tumour cell DNA. The results shown in Fig. 1*a* and *b* indicate that the tumours are clonal as they contain a limited number of integrated proviruses.

We then took advantage of the clonality of the thymomas to investigate the specificity of proviral integration in the tumour cell DNA. The specificity of integration has been extensively studied by others in a broad spectrum of cells infected with a variety of avian or mammalian retroviruses (refs 10–16 and ref. 17 for a review). These studies have dealt mostly with local specificity, and to some extent with regional or chromosomal specificity in non-tumour tissue, and have provided no evidence of preference in the choice of the site of provirus DNA integration into the host genome. We therefore postulated that the demonstration of nonrandom integration of the provirus in MoMuLV-induced tumours would strongly suggest that the insertion of the DNA provirus in certain regions of the host genome contributes to oncogenesis.

Due to multiple integrated proviruses in all tumours examined (Fig. 1*a, b*), hybridization of restriction endonuclease-digested tumour cell DNA to retrovirus DNA probes was insufficient to test the specificity of provirus integration. Therefore, we adopted the approach of cloning provirus–host junction fragments from two thymic lymphomas. The cellular DNA-specific sequences of these clones were used as probes to detect a common integration substrate in restriction endonuclease-digested DNA from 16 independent thymomas.

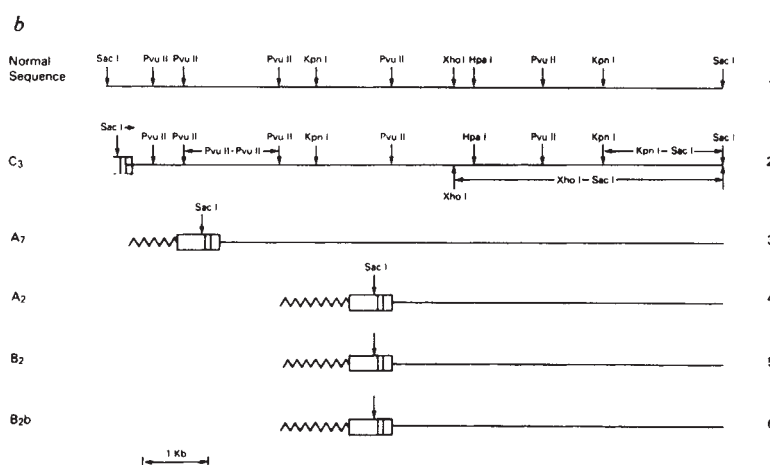
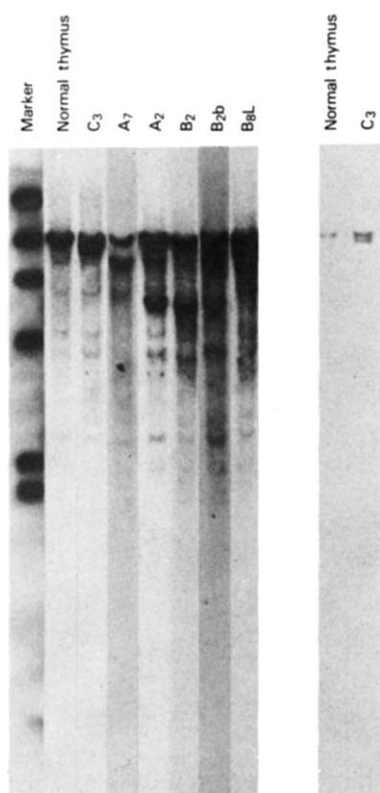
The choice of the tumours from which the provirus–host junction DNA fragments were cloned was based on an analysis of tumour cell RNA for virus–host hybrid mRNA transcripts. Such mRNA transcripts have been found, as previously predicted^{18–20}, in most cases of avian leukosis virus (ALV)-induced bursal lymphomas, and they have been shown to contain sequences of a known oncogene (*c-myc*)^{4–7}. In this instance, a regional specificity of proviral DNA integration in the vicinity of the *c-myc* gene has been shown^{4–7}.

Analysis of tumour cell RNA from 20 tumours by hybridization to the U₅ probe or the representative MoMuLV probe (Fig. 2*b*) revealed two mRNA transcripts that are likely to contain cellular and viral sequences: (1) a 2.4-kilobase (kb) mRNA transcript that hybridizes only to the U₅ probe (U₅⁺) and appears in 40% of the tumours, and (2) a 7-kb transcript that hybridizes only to the representative probe (U₅⁺) and appears in 35% of the tumours. Three out of 20 thymomas have both, while 9 out of 20 tumours have neither of the two hybrid mRNA transcripts (P.N.T., T. Wood, P.G.S. and L.F.H., unpublished). If, as in the case of the ALV-induced bursal lymphomas, these mRNA transcripts are involved in oncogenesis, the heterogeneity with respect to the appearance of hybrid mRNA transcripts would suggest alternative mechanisms of oncogenesis in the rat thymomas. We therefore cloned the provirus–host junction DNA fragments from one tumour that contained both hybrid transcripts (B8L) and one tumour that contained neither (C₃). Analysis of the RNA of the two tumours selected is shown in Fig. 2*a*. The present report gives results of the analysis of the DNA from 16 independent tumours using a cellular DNA probe derived from the provirus–cell junction DNA fragment C1C₃1A cloned from tumour C₃.

Tumour cell DNA from the rat thymoma C₃ was digested with *Sac*I (Fig. 1*d*) and analysed by hybridization to the long terminal repeat (LTR) probe or to the representative (rep) MoMuLV probe. The results shown in Fig. 1*c* identify 14 putative provirus–cell junction fragments which would suggest at least seven separate integration events in the C₃ tumour cell DNA. The 9.5-kb junction fragment was cloned into the *Sac*I site of the bacteriophage λ vector λ gtWES. λ B (ref. 21) using

Fig. 3 Identification of a common region for proviral DNA integration in MoMuLV-induced thymic lymphomas. *a*, 20 μ g of *Sac*I-digested cell DNA from normal rat thymus and six MoMuLV-induced rat thymic lymphomas was electrophoresed in a 0.8% agarose gel, Southern blotted and hybridized to the *Xho*I-*Sac*I DNA fragment from *CIC*₃1A shown in *b*. In five tumours, *C*₃ to *B*₂b, a major DNA band appears that is absent from normal rat thymus DNA. This is in addition to the 9.7-kb major DNA band and several faint bands that appear to be present in the normal DNA. The size of the additional band differs among tumours. In tumour *C*₃ it is 9.5 kb, which equals the size of the insert DNA in *λCIC*₃1A. In tumour *A*₇ it is 7 kb and in tumours *A*₂-*B*₂b it is 5.7 kb. In tumour *B*₈L, as well as 10 additional tumours that are not shown in this figure, the hybridization pattern seems to be the same as in the normal rat thymus DNA. When *Sac*I-digested DNA from the same tumours was hybridized to the LTR probe (Fig. 1*b*) we could identify a fragment of 9.5 kb in tumour *C*₃ and a fragment of

7 kb in tumour *A*₇. In tumours *A*₂, *B*₂ and *B*₂b the 5.7-kb fragment cannot be seen because it is obscured by the 5.5-kb internal provirus band. The 9.7- and 9.5-kb bands in tumour *C*₃ can be distinguished only in lightly exposed films. This is shown in the right-hand side of *a*, where a short exposure of the autoradiogram of the normal rat thymus and tumour *C*₃ lanes is presented. The marker is *Hind*III-digested λ DNA. *b*, Interpretation of the data in *a*. Line 2 shows a restriction map of *CIC*₃1A with all the restriction sites that are important for the present study. Line 1 shows the expected restriction map of the normal cellular DNA sequence represented by *CIC*₃1A before provirus integration (assuming that the integration event does not stimulate deletions or rearrangements in the cellular DNA). Finally, lines 2-6 show the expected sites of provirus integration within this cellular DNA sequence in tumours *C*₃, *A*₇, *A*₂, *B*₂ and *B*₂b. The orientation of the provirus in tumours *A*₇, *A*₂, *B*₂ and *B*₂b, although consistent with the data, has not been verified by hybridization to the *U*₅ or *U*₃ MoMuLV probe. There is no evidence for provirus integration within this sequence in tumour *B*₈L and 10 additional tumours we have examined.



an LTR probe to screen for positive phage plaques. DNA from *λCIC*₃1A was digested with *Sac*I and analysed by hybridization to the *U*₅, the *U*₃ and the rep MoMuLV probe shown in Fig. 2*b*. The results shown in Fig. 1*c* indicate that *CIC*₃1A represents a junction fragment that extends from the 3' LTR of the integrated provirus into the flanking cellular DNA.

A nick-translated DNA probe extending from the *Xho*I to the *Sac*I site of the *CIC*₃1A DNA (Fig. 3*b*) was used to hybridize to *Sac*I-digested normal thymus and tumour cell DNA. The results are shown in Fig. 3*a*. A strongly hybridizing fragment at 9.7 kb and several less prominent fragments were identified in the normal thymus cell DNA. Five out of the 16 tumours examined showed an additional fragment of variable size hybridizing to this probe. We conclude from this experiment that the *CIC*₃1A cellular DNA serves as a substrate for MoMuLV provirus integration in many tumours. There was no correlation between the presence of an integrated provirus within this sequence in the tumour cell DNA and the presence of hybrid virus-host mRNA transcripts in the same tumour. The site of integration, based on the size of the fragments we detected in the cellular DNA in the five tumours, is shown in Fig. 3*b*.

To exclude the possibility of polymorphism in this region among individual rats, the following experiments were performed.

(1) Cellular DNA from 16 tumours was digested with *Kpn*I which cleaves within the R region of the MoMuLV LTR^{22,23}. The digested DNA was analysed by hybridization to the *CIC*₃1A *Pvu*II-*Pvu*II probe shown in Fig. 3*b*. If our explanation of the integration pattern of MoMuLV shown in Fig. 3*b* is correct, we would expect that tumours *A*₂, *B*₂ and *B*₂b would not show

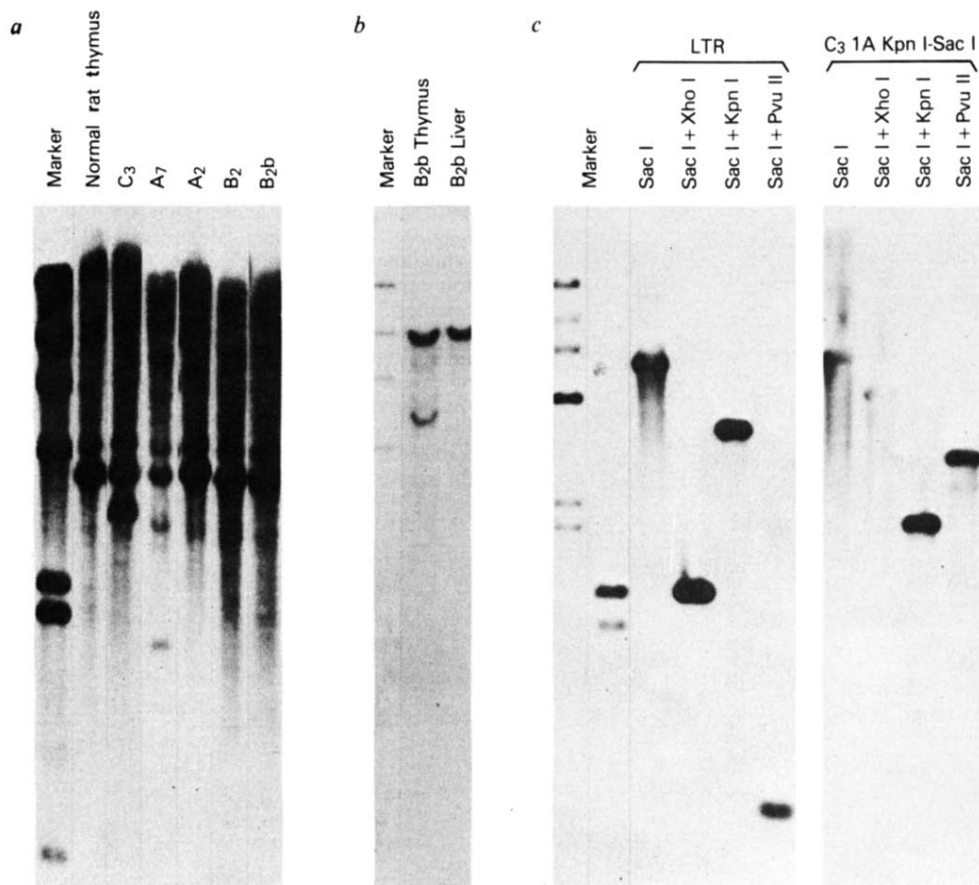
any DNA rearrangement by this analysis. However, tumour *C*₃ would give an additional 3.5-kb fragment and tumour *A*₇ would give a 1.7-kb fragment. The results in Fig. 4*a* agree with this prediction.

(2) The liver of rat *B*₂b was not involved in tumour metastasis. The DNA derived from the thymoma and the non-involved liver of the same animal were both digested with *Sac*I and analysed by hybridization to the *Xho*I-*Sac*I *CIC*₃1A cellular DNA probe. The results shown in Fig. 4*b* indicate that the additional 5.7-kb fragment is seen only in the tumour and not in the liver cell DNA.

(3) The purpose of the next experiment was to test the hypothesis that the MoMuLV LTR and the cellular sequences in *CIC*₃1A were covalently linked in the DNA of a tumour other than the *C*₃ thymoma. Therefore, the 5.7-kb rearranged *Sac*I fragment in the *A*₂ thymoma DNA was cloned into the *Sac*I site of *λ*gtWES.λB using the *CIC*₃1A *Xho*I-*Sac*I fragment as a probe. DNA from one clone we obtained (*ClA*₂1A) was digested with *Sac*I, *Sac*I + *Xho*I, *Sac*I + *Kpn*I and *Sac*I + *Pvu*II and was hybridized to the LTR probe or to the *Kpn*I-*Sac*I cellular DNA probe derived from *CIC*₃1A. The results shown in Fig. 4*c* confirm the hypothesis that the cellular DNA rearrangement in tumour *A*₂ is due to MoMuLV provirus DNA integration at the site indicated in Fig. 3*b*.

The MoMuLV provirus integration within a common domain of tumour cell DNA can be given several different interpretations. First, the MoMuLV provirus integrates randomly within the target cell DNA. However, integration within the *MLVI-1* locus initiates transformation, and the integration event is selected because it indirectly provides the cell with a powerful advantage for clonal expansion. Second, the *MLVI-1* locus

Fig. 4 The rearrangements observed in tumour cell DNA within the *MLVI-1* locus are due to provirus integration and not to genetic polymorphism in this sequence among individual rats. *a*, 20 μ g of *KpnI*-digested DNA from normal rat thymus and tumours C₃, A₇, B₂ and B_{2b} was electrophoresed in a 0.8% agarose gel, Southern blotted and hybridized to the *PvuII*-*PvuII* C1C₃1A probe (Fig. 3*b*). While DNA from tumours A₂, B₂ and B_{2b} gives the same pattern as normal rat thymus DNA, tumours C₃ and A₇ give an additional band at 3.5 kb and 1.7 kb, respectively, as expected (Fig. 3*b*). A 3.3-kb band observed in tumour A₇ probably contains cellular sequences flanking the 5' end of the integrated provirus. The marker is *HindIII*-digested λ DNA. *b*, 20 μ g of *SacI*-digested DNA from thymus and liver of rat B_{2b} was electrophoresed in a 0.8% agarose gel, Southern-blotted and hybridized to the *XhoI*-*SacI* C1C₃1A probe (Fig. 4*b*). A 5.7-kb band appears only in the DNA derived from the tumour-involved thymus and not in the DNA of the non-involved liver of the same rat. The marker is *HindIII*-digested λ DNA. *c*, Analysis of λ C1A₂1A derived from rat A₂. Cellular DNA from tumour A₂ was digested with *SacI* and fractionated by agarose gel electrophoresis. A DNA fraction containing the 5.7-kb fragment that hybridizes to the C1C₃1A *XhoI*-*SacI* probe was ligated to the *SacI* site of the bacteriophage vector λ gtWES. λ B. Phage plaques were screened using the C1C₃1A *XhoI*-*SacI* cellular DNA probe. The clone DNA was digested with *SacI* to release the insert from the λ vector DNA and also with *XhoI*, *KpnI* and *PvuII*. These enzymes should cleave the cloned DNA into fragments the size of which can be predicted from the restriction analysis of C1C₃1A. The DNA was electrophoresed in 0.8% agarose gels and hybridized to the MoMuLV LTR probe or to the C1C₃1A *KpnI*-*SacI* probe (Fig. 3*b*). The *SacI* 5.7-kb fragment hybridized to both probes. In addition, the *XhoI* digestion generated a 1.3-kb fragment that hybridized to the LTR probe and a 4.4-kb fragment that hybridized to the *KpnI*-*SacI* C1C₃1A probe. Digestion with *KpnI* generated a 3.7-kb fragment hybridizing to the LTR probe and a 2-kb fragment hybridizing to the C1C₃1A probe. Finally, the *PvuII* digestion generated a 3-kb fragment that hybridized to the C1C₃1A probe, a 2-kb fragment that was not detected by either probe, and a small fragment that hybridized the LTR probe. The size of the restriction fragments generated and their hybridization pattern confirm the hypothesis that the DNA rearrangement of the C1C₃1A sequences in tumour A₂ is due to MoMuLV provirus DNA integration into the site presented in Fig. 3*b*. The marker is *HindIII*-digested λ DNA (left lane) and Φ X174 RF DNA digested with *HaeIII* (right lane). *HaeIII* digestion of Φ X174 RF DNA generates several small-size fragments. The sizes of the two larger fragments appearing in this figure are 1.35 and 1.08 kb.



represents a substrate for preferred provirus integration in the target cell for thymic lymphomagenesis. This preferred integration event is unrelated to tumour induction. In this case the detection of the integrated provirus within this locus would simply reflect the frequent occurrence of the event. Alternatively, proviral DNA integration within the *MLVI-1* locus is both preferred and selected during oncogenesis, as has been proposed for the ALV provirus integration in the vicinity of *c-myc* in bursal lymphomas²⁴. No rigorous data are available to differentiate among these formal possibilities. However, in this system preferred integration unrelated to tumour induction seems unlikely. There are an average of 5–10 integrated proviruses in each thymoma. Analysis of 16 tumour DNAs for rearrangements within the *MLVI-1* locus revealed only 5 out of about 110 provirus integration events within this region. In addition, 10 cloned provirus–host junction DNA fragments derived from tumour B8L show no evidence that any of them represents an integration event within the *MLVI-1* locus (unpublished data). Only one previous study has suggested nonrandom provirus integration into the host genome. Baboon endogenous virus integrates into the genome of rodent–human cell hybrids only when the human chromosome 6 is present. This has been interpreted as an indication that sequences within the human chromosome 6 (the *BEVI* locus) may serve as the substrate for provirus integration²⁵. However, this has not been

proven, and the *BEVI* locus may be required in *trans* for maintenance of the integrated provirus.

While provirus integration in virus-infected non-tumour cells seems to be random, it is nonrandom in retrovirus-induced malignancies. ALV- and reticuloendotheliosis virus-induced avian bursal lymphomas carry a provirus integrated in the vicinity of *c-myc*, a known oncogene^{4–8}. Provirus DNA integration in a common domain of cellular DNA was also recently shown in mouse mammary carcinomas induced by murine mammary tumour virus (MMTV)²⁶. Formal proof that integration within a common region of cellular DNA induces tumour development is lacking in all of these systems. However, the fact that in the case of the avian bursal lymphomas the provirus integrates in the vicinity of a known oncogene suggests that common provirus integration events probably contribute to oncogenesis.

The mechanism by which MoMuLV provirus integration could contribute to oncogenesis is not yet defined. However, the absence of virus–host hybrid mRNA transcripts from tumour C₃ and the absence of correlation between integration into the *MLVI-1* locus and the presence of hybrid transcripts in other tumours suggests that provirus integration into this locus does not direct the constitutive expression of an oncogene by ‘promoter insertion’^{4–6}. Nevertheless, the provirus integration into the *MLVI-1* locus could still promote the transcrip-

tional enhancement⁷ or inactivation of neighbouring cellular genes^{27,28}. An understanding of the processes involved will require further analysis of both the viral and flanking cellular sequences involved in this integration event.

Several lines of evidence produced by our studies indicate that oncogenesis is a complex process and that provirus integration into the *MLV-1* locus may not be either necessary or sufficient for tumour induction. Provirus integration into the *MLV-1* locus appears in only 5 out of 16 tumours. This is a minimum estimate because integration events in the same locus but outside the boundaries of the *CIC₃1A* DNA fragment would not be detected with our probes. However, we cannot exclude the possibility that some tumours do not carry a provirus integrated in this region. Also, we have recently obtained evidence for an additional locus of common proviral DNA integration (*MLV-2* locus). This evidence was produced by analysis of the cloned provirus-host junction DNA fragments derived from tumour B8L. Certain tumours such as *C₃* carry proviruses integrated in both loci (unpublished data).

Although the nature of the hybrid mRNA transcripts observed in the rat thymomas has not yet been determined, it is possible that they are involved in oncogenesis. The heterogeneity of the tumours in terms of these messages and the absence of a correlation between the detection of the messages and provirus DNA integration into the *MLV-1* locus suggest that tumour induction and maintenance may result from the independent activity of either event, or complex interactions between these two phenomena.

Tumour DNA from ALV-induced bursal lymphomas²⁹, MMTV-induced mammary carcinomas³⁰ and murine B and T lymphomas induced by a variety of agents³¹ has been found to carry transforming activity detectable on transfection into NIH 3T3 cells. The activated oncogene was not linked to the integrated DNA provirus in any of the retrovirus-induced malignancies. This parameter has not yet been examined in the MoMuLV-induced thymic lymphomas. However, it is possible that the tumours studied here will be similar in this respect to the other retrovirus-induced malignancies.

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Multiple mRNA species with distinct 3' termini are transcribed from the β_2 -microglobulin gene

Jane R. Parnes* & Randy R. Robinson*

Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20205, USA

J. G. Seidman

Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115, USA

β_2 -Microglobulin is the small, relatively invariant subunit of a family of cell-surface glycoproteins encoded within the major histocompatibility complex (MHC). Proteins associated with β_2 -microglobulin in the mouse include the classical transplantation antigens (H-2K, D and L), the thymus leukaemia antigen (TL) and certain haematopoietic cell differentiation antigens (Qa-1 and Qa-2). The genes encoding these proteins are members of a large, multigene family¹⁻³. In contrast, β_2 -microglobulin is encoded by a single copy gene⁴ on mouse chromosome 2 (refs 5, 6). We have shown that this gene consists of four coding blocks separated by three intervening sequences⁴. We now demonstrate that the single β_2 -microglobulin gene is transcribed into at least two different size classes of mRNA that differ in the lengths of their 3' untranslated regions. We further show that three polyadenylation signals and a poly (A) tail are encoded at the 3' end of the gene.

Studies of β_2 -microglobulin mRNA expression in teratocarcinoma cells by ourselves⁷ and others⁸ have shown that β_2 -microglobulin specific DNA probes hybridize to at least two species of mRNA, differing in size by ~200 bases. To determine whether these mRNAs differ in protein coding or untranslated regions, we examined the protein synthesized when the two size classes of mRNA were translated *in vitro*. Poly(A)-containing RNA from a B-cell lymphoma was size-fractionated and the fractions were assayed for β_2 -microglobulin specific mRNA by blot hybridization. As shown in Fig. 1a, fractions 13 and 16 contained the large and small mRNA species, respectively. Equal quantities of these fractions were translated *in vitro* and the products were immunoprecipitated with a rabbit anti-mouse β_2 -microglobulin antiserum. Figure 1b shows that both size classes of mRNA translated into the same size β_2 -microglobulin protein. Therefore, the two mRNAs must differ, at least primarily, in an untranslated region.

We hybridized blots of C57BL/6 liver mRNA to a series of probes from different parts of the β_2 -microglobulin gene in an attempt to find the segment of the gene that encodes only one of the two mRNAs. Probes containing coding blocks I (leader) or II (amino acids 3-95) hybridized to both classes of RNA (Fig. 2). Probes containing coding block III or the 5' end of

* Present addresses: Department of Medicine, Stanford University Medical Center, Stanford, California 94305, USA (J.R.P.); Ingene, 1701 Colorado Avenue, Santa Monica, California 90404, USA (R.R.R.).

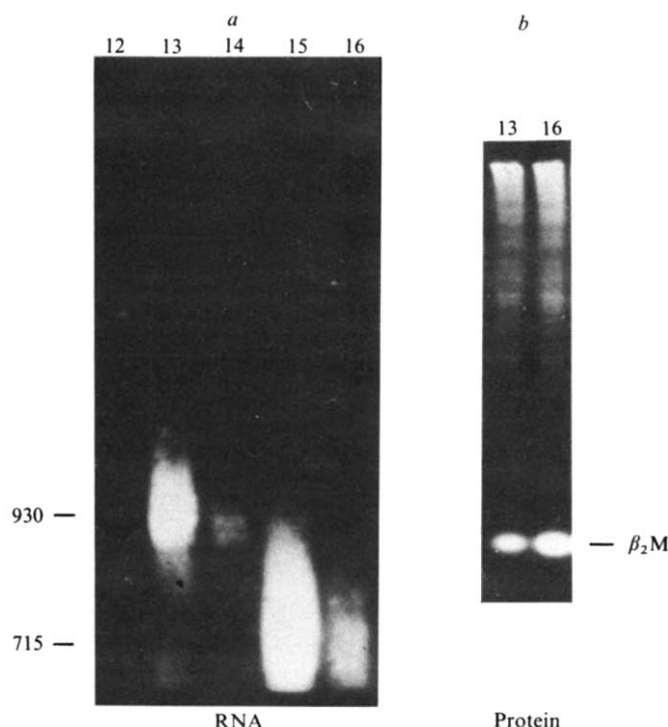


Fig. 1 Size fractionation and *Xenopus* oocyte translation of β_2 -microglobulin mRNAs. β_2 -M, position of the β_2 -microglobulin band. Polysomal poly(A)⁺ RNA was isolated from the B-cell lymphoma L10A2J¹⁷ as described previously¹⁸. 100 μ g of this mRNA were fractionated on a 1% low melting point agarose gel containing 10 mM methylmercury hydroxide and 1×E buffer¹⁹. The gel was immersed in 0.5 M ammonium acetate for 1 h at room temperature and then sliced into 1.2-mm fractions. Elution of RNA was according to Dr C. Rozek (unpublished results). Each slice was melted in 5 vol. 0.5 M ammonium acetate, 2 mM EDTA at 68 °C for 30 min. The aqueous phase was extracted three times with phenol saturated with 0.5 M ammonium acetate, then twice with chloroform/isoamyl alcohol (24:1, v/v). Carrier tRNA (10 μ g) was added, and the RNA was precipitated with 2 vol. ethanol. The RNA was collected by centrifugation, resuspended in 300 μ l 0.3 M sodium acetate pH 5, and reprecipitated with 2 vol. ethanol. After centrifugation and air-drying, the RNA from each fraction was resuspended in 10 μ l sterile water. **a**, 0.3 μ g of each RNA fraction was electrophoresed on a 2% agarose gel containing 2.2 M formaldehyde. The gel was blotted onto a nitrocellulose filter according to the procedure of Thomas²⁰. The filter was hybridized to a nick-translated 600-bp *Sst*I-*Kpn*I fragment containing coding block II of the β_2 -microglobulin gene⁴. Hybridization and wash conditions were as described previously⁴. The lanes are labelled according to the fraction of the preparative gel from which the mRNA was eluted. The sizes in nucleotides of the major mRNA species are indicated in the margin and were determined from a heat-denatured *Hinf*I digest of pBR322 run in parallel and hybridized to a nick-translated pBR322 probe. **b**, 0.12 μ g of fractions 13 and 16 were diluted to 0.08 μ g ml⁻¹ in 88 mM NaCl, 1 mM Tris pH 7.6. 0.8 μ l of either fraction was injected into eight *Xenopus laevis* oocytes. Each set of oocytes was then labelled with 100 μ Ci ³⁵S-methionine at 20 °C for 40 h in 100 μ l OR-2 buffer (ref. 21). After removal of the labelling medium, the oocytes were lysed in 200 μ l of the immunoprecipitation buffer (0.5 M NaCl, 0.5% NP-40, 10 mM Tris pH 7.6, 1% aprotinin, 0.1 mM phenylmethylsulphonyl fluoride). Nuclei and debris were removed by centrifugation. Immunoprecipitation with a rabbit anti-mouse β_2 -microglobulin antiserum was as described previously¹⁸, except that the samples were pre-cleared three times with normal rabbit serum, and the concentration of NaCl in the immunoprecipitation buffer was increased to 0.5 M. Removal of the immunoprecipitates from protein A-Sepharose beads and electrophoresis in a 12.5% SDS-polyacrylamide gel were as described previously¹⁸. The autoradiogram of the gel-fractionated immunoprecipitates is shown with the lanes labelled according to the fraction of gel-purified RNA that was translated.

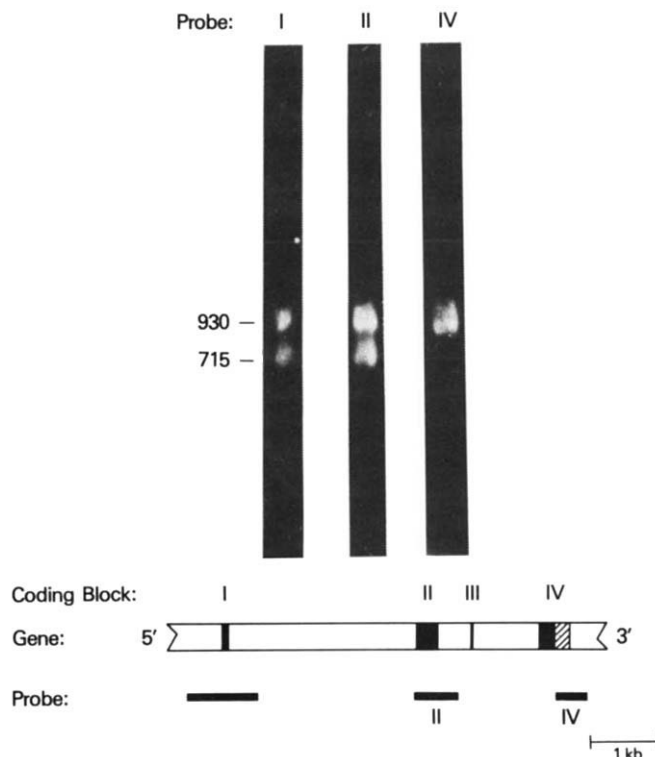
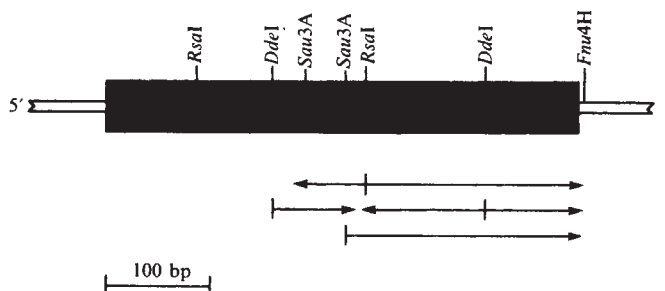


Fig. 2 Hybridization of mRNA to β_2 -microglobulin probes. The upper portion of the figure shows the autoradiogram of filter lanes hybridized to probes I, II and IV (see below). The sizes in nucleotides of the hybridizing RNAs are indicated in the margin and were determined as described in Fig. 1 legend. The lower portion of the figure shows a schematic diagram of the structure of the β_2 -microglobulin gene and the derivation of the hybridization probes. The previously identified coding blocks⁴ are indicated by the shaded boxes and represent: I, leader to amino acid 2; II, amino acids 3–95; III, amino acids 96–99 and the first 17 bp of 3' untranslated region; IV, the remainder of the 3' untranslated region. The cross-hatched portion of the coding block IV encodes the additional 3' untranslated region found here to be transcribed as part of the longest β_2 -microglobulin mRNA. The probes are numbered according to the coding blocks they contain. Probe I is a *Kpn*I-*Hind*III fragment, II is a *Sst*I-*Kpn*I fragment and IV is a *Sau*3A-*Sau*3A fragment. The 5' end of the probe IV is located 5 bp downstream of the previously identified poly(A) addition site. **Methods:** Cytoplasmic poly(A)⁺ RNA was isolated from C57BL/6 mouse liver as described previously¹⁸. The RNA (2.5 μ g per lane) was electrophoresed on a 2% agarose gel containing 2.2 M formaldehyde and blotted onto a nitrocellulose filter according to the procedure of Thomas²⁰. Individual lanes of the filter were hybridized to nick-translated restriction fragments isolated from sub-clones of the β_2 -microglobulin gene as described previously⁴.

coding block IV also hybridized to both size classes (data not shown). We had previously defined a 3' terminus for the β_2 -microglobulin gene by comparison of the sequence of the gene with that of a cDNA clone containing a poly(A) tail⁴. A polyadenylation signal (AATAAA) was found 16 base pairs (bp) 5' of the poly(A) addition site, in accordance with the postulated role of this sequence as a signal for polyadenylation. The same hexanucleotide appeared 48 bp 5' of the poly(A) addition site, suggesting the possibility of an alternative site of polyadenylation; but the short distance between these two putative polyadenylation signals did not seem sufficient to account for the difference in size between the two major classes of β_2 -microglobulin mRNA. We therefore constructed a probe from a genomic fragment with one end 5 bp downstream of the known poly(A) addition site and extending 450 bp in the 3' direction (probe IV in Fig. 2). This probe still hybridized to β_2 -microglobulin mRNA, but only to the larger species (Fig. 2). DNA

Fig. 3 Nucleotide sequence of coding block IV of the β_2 -microglobulin gene. The nucleotide sequence of the entire fourth coding block is shown. The first 241 bp were sequenced previously⁴ but are included to illustrate the locations of the three putative polyadenylation signals (the boxed AATAAA sequences). The known polyadenylation site is underlined and the 3' poly(A) tail is underscored with a broken line. The DNA sequence was determined by the method of Maxam and Gilbert²² except for that of a single *Sau*3A fragment which was subcloned in M13mp7 and sequenced by the dideoxy method²³. The sequencing strategy for the 3' half of this coding block is shown at the bottom below, while that for the 5' half has been presented elsewhere⁴.

CTCTGAAGATTTCATTGAACCTGCTTAATTACAAATCCAGTTTCTAATATGCTATACAATTTATGCACGCAGAAAGAAATAGCAATGTA
CACATCACCTCTTTATATCTTACTTTAAATGTTTTATGCATGTTTTCAAAATTGGAATATCTAGATAGCTGAGCAATAAACTCTTC
AATAAGTATTTTGATCAGAAATAATAATATAATTTAAGAACATAGTTGATCATATGCCAACCTCTGCTACTTCTCATTACTTGGAT
GCAGTTACTCATCTTTGGTCTATCACAACATAAGTGACATCTTCTTTGGTAAAGCAAGAGGCCTAATGAAGCTGTCTACTGTG
CCCAATGCTTAGCAATTCTACCCCAACCTGTGGCTACTTCTGCTTTGTTACTTTTACTAAATAAAAACTAAAAA
AAAAAATT



probes containing more 3' sequence did not hybridize to either mRNA (data not shown). These data imply that the difference between the two major size classes of β_2 -microglobulin mRNA is in the lengths of their 3' untranslated regions. The longer mRNA species must contain sequence derived from genomic DNA beyond the previously identified poly(A) addition site.

To identify the basis for the different 3' termini of these mRNAs, we determined the nucleotide sequence of the region beyond the known poly(A) addition site (Fig. 3). The nucleotide sequence revealed two notable features. First, the hexanucleotide AATAAA occurs once more, 200 bp 3' of this site. Second, 5 bp beyond this poly(A) addition signal is a sequence of 20 dA residues. These structures at the 3' end of the β_2 -microglobulin gene may be the molecular basis for the multiple mRNA species and may also suggest something of the evolution of the gene.

Poly(A) sequences have not been identified at the 3' termini of normal genes from higher eukaryotes. However, such poly(A) sequences have recently been described at the 3' ends of dispersed processed pseudogenes⁹⁻¹¹, and have been thought to provide evidence for an RNA intermediate in the mobilization of these genes from their respective loci to other chromosomal sites. Similarly, the poly(A) sequence at the 3' end of the β_2 -microglobulin gene suggests that a portion of the gene may have been mobilized via an RNA intermediate in the evolutionary past rather than having a current biological function. However, the remainder of the β_2 -microglobulin gene must have changed considerably since such an event because the gene contains features not found in dispersed pseudogenes. Namely, the gene is single copy, contains intervening sequences, and does not cross-hybridize with other genes. The suggestion^{1,12,13} that β_2 -microglobulin has evolved from the same ancestral gene as the HLA-DR molecules, immunoglobulins and H-2 molecules raises the possibility that this poly(A) sequence reflects the mechanism by which this gene has evolved from these genes.

From the sequence at the 3' end of the β_2 -microglobulin gene we have proposed a model for the structure of the mRNAs made from this gene (Fig. 4). Probably each poly(A) addition signal can lead to poly(A) addition and thus three different mRNAs could be generated. The data supporting this model are: (1) one cDNA clone has been obtained that represents a mRNA using the second polyadenylation site; (2) restriction fragments derived from sequences of 3' of this site hybridize only to the longer mRNA; and (3) the size of the longer mRNA (about 930 bases) is consistent with a poly(A) addition site in this region. The model proposes that poly(A) residues are added to the longer mRNA species in the middle of the genomically encoded poly(A) tract. The data presented here, however, do not allow us to propose the structure of the 5' end of these mRNAs. Although the 5' end of the mRNA species may be

slightly heterogeneous, the hybridization data presented in Fig. 2 suggest that the major size heterogeneity is due to differences in the 3' ends of the RNA species.

Although most sequenced eukaryotic genes contain a single poly(A) addition site, multiple sites have been described in the genes encoding mouse dihydrofolate reductase¹⁴, α -amylase Amy-1¹⁵ (ref. 15), and immunoglobulin heavy chain μ (ref. 16). In one case, the immunoglobulin heavy chain mRNAs, these multiple poly(A) addition sites seem to have an important role in determining the function of the protein encoded by a particular RNA. However, the biological function of 3' untranslated regions is unknown, and thus the significance of variations in their length is unclear. We have examined mRNA from a variety of cell types (liver, myeloma, teratocarcinoma, T and B cell lymphoma lines, salivary gland, spleen and fibroblasts) by blot hybridization but have been unable to detect any major tissue-specific differences in the relative distribution of the two major size classes of β_2 -microglobulin mRNA. Nevertheless, it is possible that the differences in the 3' untranslated regions in some way affect the subcellular compartmentalization of the mRNA and consequently the fate of the translated protein. For example, protein translated from one species might be secreted from the cell while that from another could be directed to associate with one of the MHC-encoded proteins. Perhaps these multiple poly(A) addition sites have a role in allowing the single β_2 -microglobulin gene to service the large number of H-2-like genes. Eventually, *in vitro* alteration of the β_2 -microglobulin gene and subsequent expression in mouse cells should lead to a better understanding of the function of the multiple species of mRNA.

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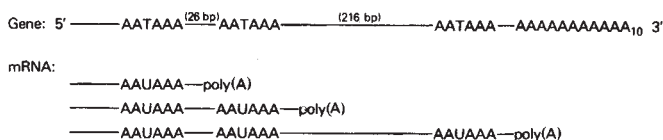


Fig. 4 Model for derivation of β_2 -microglobulin mRNAs with distinct polyadenylation sites. A representation of the 3' end of the β_2 -microglobulin gene is shown at the top. The three polyadenylation signals (AATAAA) and the poly(A) tail of 20 dA residues are shown. The numbers in parentheses indicate the spacing between the polyadenylation signals. Three possible 3' termini for polyadenylated β_2 -microglobulin mRNA are shown at the bottom. Each makes use of a different polyadenylation signal (AAUAAA). A cDNA clone corresponding to the middle RNA species has been sequenced⁴ and evidence for the longest species is presented. The smallest species has not been identified to date.

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Use of an ¹²⁵I-labelled DNA ligand to probe DNA structure

Roger F. Martin & Noni Holmes

Biological Research Unit, Cancer Institute, 481 Little Lonsdale Street, Melbourne, Victoria 3000, Australia

It no longer seems likely that DNA molecules *in situ* have a uniform conformation, represented by the classical B-form helix. For example, recent structural studies have shown that in certain conditions DNA can have a left-handed (so-called Z-form) helix¹, and have revealed extensive sequence-dependent variations of B-DNA helical parameters². Such sequence-dependent variations in DNA structure can be investigated in solution with reagents that bind to DNA in a conformation-dependent manner, and cut one or both strands of the double-helix at the site of binding, as, for example, has been shown for the endonuclease DNase I³. We describe here a simple way to endow a DNA-binding ligand with the ability to cleave DNA—labelling with ¹²⁵I. The radiochemical damage associated with ¹²⁵I decay induces a double-stranded DNA break. Using this technique we have shown that a sequence of four consecutive A·T base pairs is a necessary, but not sufficient, condition for strong binding to DNA of the *bis*-benzamide Hoechst 33258—presumably the other important factor is the conformation of the double-helix at the site of the (A/T)₄ sequence. We suggest ¹²⁵I-Hoechst 33258 may be a useful new probe of DNA structure.

The radiochemically damaging properties of ¹²⁵I decay are thought to result from the emission of very low energy (Auger) electrons, of which an average of 22 are emitted per decay⁴. However, it is not clear whether it is the electron irradiation, or the consequent positive charge dissipating from the daughter ¹²⁵Te atom, that is the most damaging feature of the decay event⁵. In any case, it is well established that each decay in ¹²⁵I-labelled DNA results in a double-stranded DNA break⁶. The extent of damage was recently evaluated in DNA segments of defined sequence containing a single ¹²⁵I-labelled deoxycytidine in a unique location, and the results indicated that each ¹²⁵I decay results in multiple scissions in each DNA strand, with the majority of damage confined to within about 4 base pairs (bp) of the decaying atom⁷.

The ¹²⁵I atom does not need to be covalently bound to DNA to cause cleavage, ¹²⁵I-labelled aminoacridines also induce double-stranded breaks when bound to DNA^{8,9}. Thus, introduction of an ¹²⁵I atom into a DNA ligand will be expected

Table 1 Nucleotide sequences of cleavage hotspots

4,175	aaTATTat	vs	4,199	gtTATTgt	m
4,221	caTATTtg	s	4,150	tgAATAct	m
4,231	tgTATTta	s	4,305	caTTATta	m
57	caATTTaa	s'	4,267	acATTTcc	m
88	agATTTca	s'	4,276	cgAAAAgt	w
4,142	ggAAATgt	s			
4,240	aaAAATaa	s	45	gaTAAAct	vw'
4,248	acAAATag	s	34	gcATTAaa	vw'
4,188	caTTTAtc	s	4,318	acATTAac	vw
4,167	ccTTTTtc	s	4,206	tcTCATga	vw
4,150	tgAATAct	m	4,297	taAGAAac	vw
4,329	taAAAAta	m	4,255	ggGGTTcc	vw

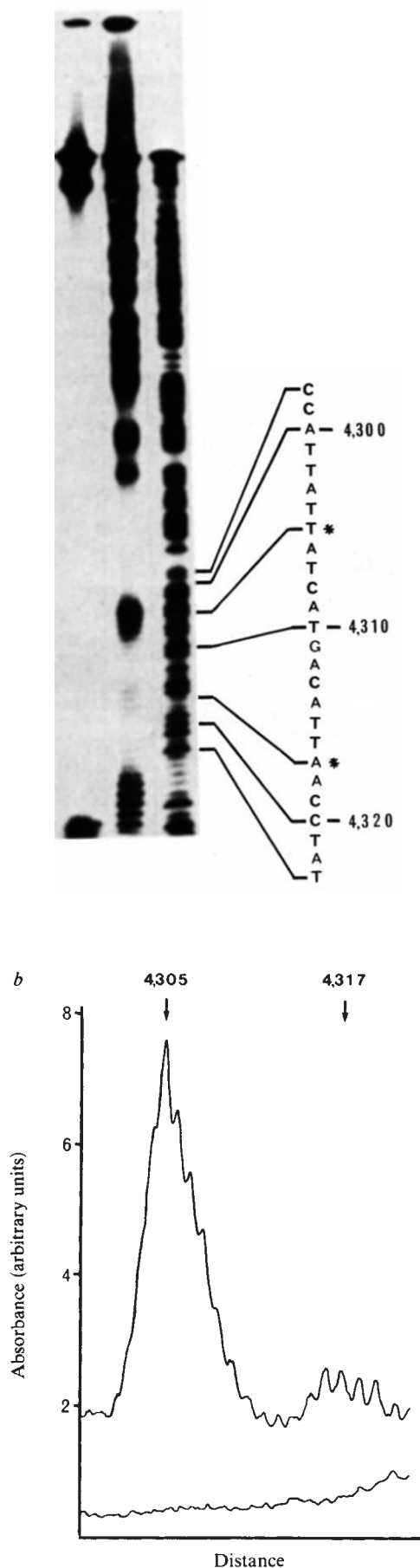
The four capital letters in bold type denote the tetranucleotide sequence (5'–3') of the presumed binding sites. The lower case letters show the neighbouring nucleotides. The underlined letter denotes the nucleotide at, or within one base pair of, the centre of the hotspot (Fig. 2), and is identified by the base pair number in the pBR322 sequence reported by Sutcliffe¹⁶. The bold lower case letters indicate the relative intensities of the hotspots, as determined from densitometer scans of autoradiographs. Average peak heights of >1.0, 0.7–1.0, 0.3–0.7, 0.15–0.3 and <0.15 relative to that of hotspot at position 4,221 are denoted by vs, s, m, w and vw, respectively. s' and vw' denote average peak heights of 0.7–1.0 and <0.15 relative to that of the hotspot at position 57.

to confer an endonuclease-like activity. Moreover, if the labelled ligand interacts with a DNA fragment of defined sequence, the sites of cleavage can be mapped by the application of DNA sequencing techniques, as used in sequence selectivity studies of a number of DNA ligands with intrinsic endonuclease activity^{10–14}. Accordingly, we have prepared ¹²⁵I-labelled Hoechst 33258 and now report the sequence selectivity with fragments of DNA from pBR322.

Hoechst 33258 is a *bis*-benzamide derivative containing a terminal phenolic hydroxyl. The compound is easily iodinated in the conditions used routinely for ¹²⁵I-labelling of protein tyrosines. In this study we have used the Iodogen technique¹⁵. The reaction conditions used a large excess of the substrate in the presence of carrier-free ¹²⁵I-iodide, and the predominant product was presumably the mono-*o*-substituted derivative, which was purified by preparative TLC.

As target molecules we used a 346 bp *EcoRI*–*TaqIII* fragment, and a 176 bp *EcoRI*–*HaeIII* fragment, each derived from pBR322 (which has been completely sequenced¹⁶), and each 3'-labelled at the *EcoRI* end with ³²P. After incubation with ¹²⁵I-labelled Hoechst 33258, the sample was treated to remove most of the ¹²⁵I, which was present in large excess over the ³²P, and loaded onto a DNA sequencing gel. A typical result is shown in Fig. 1. 'Hotspots' are apparent in the sample incubated with the ¹²⁵I-labelled ligand (lane 2, Fig. 1a), but not for the samples in which the ³²P-labelled target DNA was incubated in buffer alone (lane 1). In similar experiments, control incubations containing an equivalent amount of ¹²⁵I as iodide, or very low specific activity ¹²⁵I-Hoechst 33258, were also free of hotspots (data not shown). A densitometer scan of one of the hotspots (Fig. 1b) shows it to be comprised of a family of several bands with an intensity bias to the 3' end of the DNA. Both the range and asymmetry of damage are consistent with the earlier study⁷. The apparent bias of the damage towards the ³²P-labelled end is the major evidence that each ¹²⁵I decay induces multiple scissions in each DNA strand. The densitometer scan also illustrates the marked difference in intensity between binding/damage at sites 4305 and 4317.

We have analysed seven sequencing gels of the kind shown in Fig. 1, and two gels from similar experiments using the *EcoRI*–*HaeIII* fragment from pBR322. Each hotspot consists of five or six bands that are clearly obvious above background. The location of the centre of each hotspot relative to the known nucleotide sequence of pBR322¹⁶ was determined by reference to DNA sequencing tracks¹⁷, which were generated from the



respective 3' ^{32}P -labelled pBR322 fragments. The precision of locating the centre of the hotspots by this procedure is approximately ± 1 bp. The results summarized in Fig. 2 show that the location of each hotspot is generally reproducible between experiments. The major exception was the site at 4,199, which was detected as a medium intensity hotspot in two gels (∇ , $\blacktriangledown$) but not in another experiment (Δ), in which a weak hotspot was seen at 4,206. A 'consensus' sequence for ^{125}I -Hoechst 33258 binding is evident; the centre of each hotspot is adjacent, or nearly adjacent (± 1 bp) to four consecutive A·T base pairs (Table 1). The relative intensity of each of the hotspots was obtained from densitometer scans and listed in Table 1. The only exceptions to the consensus are three very low intensity hotspots at sites 4,206, 4,255 and 4,297. However if four consecutive A·T base pairs are necessary to determine a binding site, that condition is not always sufficient—some potential binding sites (for example, 34, 45, 4318) are only very weakly cut.

The finding that the binding site for ^{125}I -Hoechst includes the sequence (A/T)₄ is consistent with a recently reported model of binding of Hoechst 33258 to B-DNA¹⁸. Our own model building studies have confirmed the general features of that model. The two amine groups, and one methyl amine of the Hoechst dye can hydrogen bond to adenine and thymine bases in the minor groove of the double-helix. Of the 10 possible different ways of arranging 4 consecutive A·T base pairs, all have at least one possible configuration for forming a minor groove complex with Hoechst 33258 involving three hydrogen bonds. No doubt the many different configurations are associated with a spectrum of probabilities of formation, and more data of the sort reported here would enable some ranking of the different tetranucleotides according to their affinity for Hoechst 33258.

Our model-building studies also showed that for most (A/T) tetranucleotides, Hoechst 33258 could bind with either polarity, suggesting that two hotspots would be expected for each binding site. However, it is clear from Fig. 2 that we have detected only one hotspot, on the ^{32}P -labelled, 3' side of each tetranucleotide binding site. We believe that the apparent anomaly can be explained as follows. Assuming that each ^{125}I decay results in multiple strand breaks in both strands, then there will be a distribution of strand-break probability associated with each ^{125}I location in the ^{125}I -Hoechst 33258 DNA complex. We only

Fig. 1 Sequencing-gel analysis of DNA fragments resulting from the incubation of ^{125}I -Hoechst with a 3'- ^{32}P -labelled *Eco*RI-*Taq*I restriction fragment of pBR322. *a*, Autoradiograph (13 day exposure) of a DNA sequencing gel¹⁷ loaded with samples of ^{32}P -DNA after incubation at 5 °C with buffer only (lane 1) or with ^{125}I -Hoechst (lane 2). Lane 3 is a Maxam and Gilbert C+T reaction mixture derived from the same ^{32}P -DNA. *b*, Densitometer-scan of portion of lanes 1 and 2 of *a*, showing the location of hotspots relative to the appropriate nucleotide sequence.

Methods: pBR322 DNA was cut with *Eco*RI and the 3'-ends labelled with [α - ^{32}P]dATP using the Klenow fragment of *Escherichia coli* DNA polymerase I. The plasmid was then cut with *Taq*I and the labelled 376 bp fragment¹⁶ was isolated by preparative polyacrylamide gel electrophoresis. Hoechst 33258 (Calbiochem) was iodinated using Iodogen¹⁵ and carrier free Na^{125}I (Amersham). The benzimidazole was in vast excess, so the mono-substituted compound was the predominant product, and was purified by preparative TLC on silica gel plates (Merck). Typical incubation mixtures contained 3'- ^{32}P -labelled pBR322 DNA fragment (10^5 c.p.m.), sonicated calf thymus DNA (1 μg), ^{125}I -Hoechst (3×10^7 c.p.m.) in 100 μl of a buffer containing 5% methanol, 0.5 M NaCl, 0.2 M Tris-acetate pH 7.0, 5 mM EDTA and 5 mM KI. After incubation at 5 °C for 3 weeks, fragmented DNA was recovered by ethanol precipitation. Each sample was mixed with 5 μg carrier DNA and subjected to alkaline hydrolysis in 0.25 M NaOH in 90% methanol for 1 h at 37 °C. After neutralization with 1 M acetic acid and ethanol precipitation, the alkaline hydrolysis step was repeated. The two cycles of alkaline hydrolysis typically reduced the ratio (in c.p.m.) of ^{125}I : ^{32}P from approximately 10–20 to approximately 1 or less.

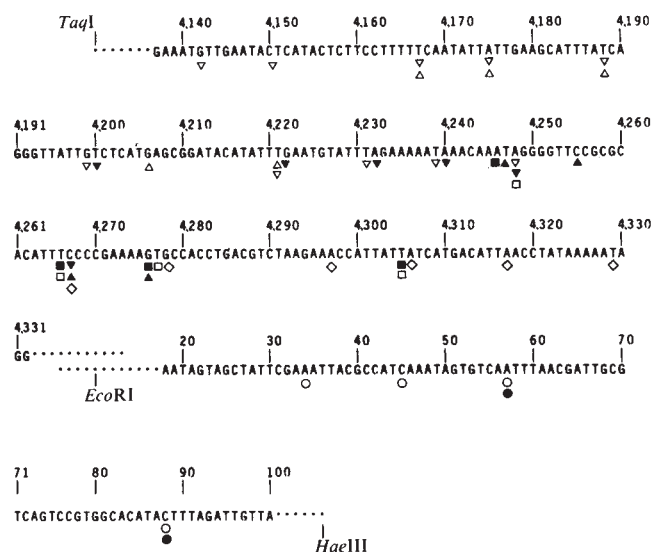


Fig. 2 Summary of ^{125}I -Hoechst 33258 binding/breakage sites on portions of the *EcoRI*-*TaqI* and *EcoRI*-*HaeIII* fragments of pBR322. The numbers correspond to the base pair numbering of the 4,362 bp pBR322 sequence as described by Sutcliffe¹⁶. The symbols indicate the centres of hotspots on sequencing gel autoradiographs. Each symbol represents a different experiment. The regions examined in each experiment were: ○, ●, 28–100; ▽, 4,136–4,253; △, 4,160–4,225; ▼, 4,192–4,268; ▲, 4,242–4,290; □, ■, 4,242–4,310; ◇, 4,260–4,331.

have indirect information about the details of each probability distribution, and that⁷ suggests that the majority of breaks occur within 3–4 bp on either side of the decaying atom. Thus, for each tetranucleotide binding site for ^{125}I -Hoechst 33258 we would expect two such distributions, each corresponding to one of the alternative polarities of ^{125}I -Hoechst 33258 binding and centred either side of the tetranucleotide binding site. The composite of these two distributions would be centred near (depending on the relative contributions of the binding configurations) the middle of the binding site. Apparently most DNA strands in the population of ^{125}I -Hoechst-DNA complexes sustain more than one break per ^{125}I decay in the vicinity of a binding site. However, only the shortest ^{32}P -labelled fragments are represented in the distribution detected as the hotspot, and hence the marked bias toward the ^{32}P -labelled side of the binding site. There are two immediate consequences of this interpretation. The first is that if the experiment were repeated using $5'$ ^{32}P -labelled target fragments, a new collection of hotspots should be detected, corresponding to a displacement of about 5 bp from those in Fig. 2. Second, detailed examination of the actual distribution of the ^{32}P label in the population of fragments in a given hotspot should allow the generation of strand-break probability distributions by computer modelling. We are now investigating both of these avenues.

While the finding that each hotspot is associated with an (A/T) tetranucleotide is interesting *per se*, the most intriguing aspect of the results is that some potential sites of four consecutive A·T base pairs do not bind significant amounts of the labelled ligand. Zimmer *et al.*¹⁹ have recently reported the use of netropsin, which binds to the minor groove of B-DNA but does not bind significantly to Z-DNA or A-DNA, as a specific probe for B-DNA. If I-Hoechst 33258 has as marked a preference for B-DNA as uniodinated Hoechst 33258¹⁸, then it is tempting to speculate that the potential binding sites that show low affinity are in another than B-conformation. In any case, it is reasonable to suggest that the observed patterns of binding/damage are a result of both the affinity of the probe for a 'consensus' sequence, and regional perturbations in conformation along the DNA helix. Such structural features have been detected recently with a phenanthroline-copper (II) com-

plex^{20,21}. Note that although our experiments were performed at very low ligand:DNA ratios, the possibility that the I-Hoechst 33258 cooperatively induces conformational changes in the target DNA cannot be excluded. Such effects are likely to complicate the interpretation of the results from another recently reported method for investigating sequence selectivity of DNA ligands, based on patterns of inhibition of cleavage by methidium-EDTA·Fe(II)²².

We suggest that ^{125}I -Hoechst and other similarly labelled DNA ligands may be useful as probes to investigate variations of conformation in relatively long DNA molecules which at present are beyond the reach of analysis by X-ray crystallography. Moreover, probes such as ^{125}I -Hoechst 33258 do not rely on an enzyme activity, and can be used in the relatively extreme conditions which are known to induce conformational changes in DNA. Finally, we should point out that our results also demonstrate an approach for investigating the sequence selectivity of any DNA ligand which can be labelled with ^{125}I .

We thank Dr G. S. Hodgson, who initially suggested the Hoechst *bis*-benzimidazoles as candidate DNA ligands for iodination, for his continuing support and advice, and Dr L. P. G. Wakelin for his advice on model building.

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Errata

In the article 'Deuterium excess in an East Antarctic ice core suggests higher relative humidity at the oceanic surface during the last glacial maximum' by J. Jouzel, L. Merlivat and C. Lorius, *Nature* **299**, 688–691 (1982), the address given for J. Jouzel and L. Merlivat is incorrect. The address should read: Laboratoire de Géochimie Isotopique, DPC, Centre d'Études Nucléaires de Saclay, 91191 Gif-sur-Yvette Cedex, France.

In the letter 'Overlapping spreading centres: new accretion geometry on the East Pacific Rise' by K. C. Macdonald and P. J. Fox, *Nature* **302**, 55–58 (1983), on pages 56 and 57 the legends of Figures 2 and 3 were transposed.

Corrigenda

In the letter 'Evidence for $^{13}\text{C}/^{12}\text{C}$ fractionation between tree leaves and wood' by S. W. Leavitt and A. Long, *Nature* **298**, 742–744 (1982), on page 743 the monthly Tucson temperatures in the first line of the second column are incorrect. The correct temperatures are: 22.8, 24.4, 30.4, 30.3, 31.5 and 28.8 °C. The temperature and correlation coefficients were correct.

In the article 'Activation of a cellular oncogene by DNA rearrangement: possible involvement of an IS-like element' by G. Rechavi, D. Givol and E. Canaani, *Nature* **300**, 607–611 (1982), the acknowledgement on page 610 should have indicated support from the Edith C. Blum Foundation, not the Blum Foundation.

Geomagnetic excursions and climate change

KENT¹ has demonstrated rather convincingly that the intensity of natural remanent magnetism (NRM) in deep-sea sediments is sensitive to changes in sediment type, and hence is not an accurate indicator of the true strength of the geomagnetic field. Thus, reported correlations between NRM intensity and climatic parameters in deep-sea cores may be largely or entirely a function of climatically induced changes in core lithology.

This explanation is not, however, directly relevant to suggested correlations between climate change and other aspects of the geomagnetic field. For example, excursions of the Earth's magnetic field, as recorded in sediments, have been correlated with climate fluctuations and with variations in the eccentricity of the Earth's orbit²⁻⁴. These excursions appear to represent changes in magnetic inclination and/or declination and do not necessarily involve variations of NRM intensity. The recorded excursions are not readily explained by changes in lithology and magnetic mineral content, although some workers have suggested that excursions in sediment cores are the result of disturbances of the deposits before, during or after sampling^{5,6}. The discovery of several of the excursions in non-sedimentary materials, such as lava-flow sequences and ocean-floor magnetic striping, lends support to the idea that these events were real fluctuations of the geomagnetic field⁷⁻⁹.

The proposed connection between geomagnetic excursions and climate may come about through a direct cause and effect relationship involving fluctuations in field strength that can accompany reversals and excursions^{2,10}, atmospheric effects of variations in geomagnetic pole position¹¹, or perhaps some other mechanism¹². It is possible that excursions and climate are only secondarily related and that orbital variations are the driving force for both phenomena. Seven of the reported excursions during the last 700,000 yr seem to correspond to peaks in the approximately 100,000-yr eccentricity cycle¹³. These excursions are sometimes, but not always, correlated with changes in NRM intensity in cores². Therefore, although Kent's work casts doubt on the significance of correlations of climate with NRM measurements of field strength, it does not offer an alternative explanation for the proposed connections between excursions, climate and orbital parameters.

MICHAEL R. RAMPINO

NASA, Goddard Institute
for Space Studies,
New York 10025, USA

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KENT REPLIES—Rampino is correct in stating that, unlike NRM intensity fluctuations observed in some cores¹, anomalous NRM directions interpreted as geomagnetic excursions are not readily explained by changes in lithology and magnetic mineral content. The question of excursions, particularly their temporal and spatial distribution, can be argued at length (see, for example, ref. 2). Here I will simply illustrate some of the associated problems by considering the record of purported excursions in a single critical core, V20-108, which provides an important basis for several contributions seeking a link between geomagnetism and climate³⁻⁵.

Although Wollin *et al.*⁵ are sometimes cited^{3,4} for the palaeomagnetic record of V20-108, the original data were published in an early and now classic paper on magnetic stratigraphy by Ninkovich *et al.*⁶. Comparison of the original inclination record⁶ with later versions of the same data³⁻⁵ shows some important discrepancies relevant to this discussion—for example, there are only three intervals of anomalous negative inclination, all in the upper 300 cm of the core (see Fig. 7 of ref. 6), rather than five intervals, extending to 500 cm (for example, Fig. 1 of ref. 4).

A closer look at the original data reveals that the inclinations below 300 cm in the 1,671-cm long core fall close to the expected dipole value of 63.5°, positive for normal and negative for reversed polarity. This indicates that the geomagnetic field is accurately recorded in this section of the core and supports the correlation of the reversals to the geomagnetic polarity time scale (for example, the 0.73-Myr Brunhes/Matuyama at 792 cm)⁶. In contrast, within the upper 300 cm, representing about 40% of the Brunhes, the inclinations are anomalous in two respects: first, regardless of sign, the inclinations are invariably shallower than the dipole value; and second, intervals of negative inclination occur. These intervals of anomalous negative inclination (or their facsimiles in ref. 5) have been seized upon as records of geomagnetic excursions and as such used to make various correlations with the Earth's climate or eccentricity of orbit³⁻⁵.

I argue that these intervals, and in fact the magnetic data from the entire upper 300 cm of V20-108, are unlikely to be a representation of the geomagnetic field. In two nearby cores—V20-107 taken 227 km to the south and V20-109 taken only 208 km to the north of V20-108—the magnetic data are of high internal consistency and no such anomalous inclinations are observed in the Brunhes⁶. Such localized occurrence of large departures from an axial dipole field direction is difficult to explain with sources in the Earth's core and is more likely attributable to a distorted record. Indeed, analysis of the Lamont coring and curatorial logs for V20-108 reveals that at least the upper 300 cm of the sediment was drawn through a bent pipe during coring, a condition that could easily lead to disturbance of the sediment and the magnetic record. The same logs moreover show that the sediment core was broken in several places on board the ship, in particular at 298 cm to facilitate transport and at 460 cm where coring pipes were uncoupled; single-sample measurements of anomalous magnetic inclination closely correspond to these levels. In view of these observations, the interpretation of anomalous inclinations in V20-108 as records of geomagnetic field excursions and their correlation to global phenomena is highly dubious.

This illustration is not meant to imply that all geomagnetic excursions or short-polarity intervals reported in the literature are artefacts of poor or distorted records; several, such as the Mono Lake excursion recorded in sediments^{7,8} and the Cobb Mt microchron in lavas^{9,10}, appear to be well documented. Rather, this unfortunate choice of data suggests that a more critical and conservative attitude should be taken in the documentation of geomagnetic field behaviour, considering the numerous and more mundane sources of anomalous palaeomagnetic data. A much improved knowledge of geomagnetic excursions and short-polarity intervals is required not only to provide a legitimate basis for comparison with the far better established record of Pleistocene climate change but also to gain a more fundamental understanding of the entire spectrum of geomagnetic phenomena¹¹.

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D. V. KENT

Lamont-Doherty Geological Observatory
and Department of Geological Sciences,
Columbia University,
Palisades, New York 10964, USA

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Female choice in widowbirds

FIELD studies of the long-tailed widow-bird, *Euplectes progne*, by Andersson¹ involved experimental manipulation of the tail-lengths of territorial males and measurement of their breeding success in terms of the number of females that nested in the male's territory. The Darwin–Fisher theory^{2,3} of the role of sexual selection in the evolution of bird plumage would predict that females should prefer to mate with males with longer tails. Although Andersson interprets his data as supporting this prediction, we feel that this may not be justified in view of the following oversights.

First, the number of new nests was not least for males with shortened tails as claimed, there being no difference between males with short tails and normal males (four new nests in each case). Second, Andersson claims¹ that any tendency of females to mate in one territory but nest in another (which is known to occur in other polygynous birds⁴) would fail to produce a spurious bias in favour of the Darwin–Fisher theory. This claim is only justified, however, if the Darwin–Fisher theory is true and females prefer to mate with long-tailed males but nest in the territories of other males. If the theory is not true, and females mate randomly or with a preference for short-tailed males but nest in the territories of long-tailed males, such behaviour could produce a spurious bias in the results in favour of the theory.

Third, over the range of tail lengths that sexual selection would be assumed to have acted in the evolutionary past (that is, short to normal tail), there is no hint that females prefer longer tails (for example, no correlation between tail length and number of nests on the territory before treatment began; absolutely no difference between short-tailed and normal males; and a tendency to prefer normal-but-cut to normal males even though the tails of the former were on average 1 cm shorter).

Males with long tails did significantly better than normal males and males with shortened tails, but did not do significantly better than males with normal-but-cut tails. The proper conclusion from the data should therefore have been that females preferred to nest in the territories of males with 25 cm of feathers glued on to them, irrespective of the total tail length. Such a conclusion could be interpreted in terms

of predation hypotheses for the evolution of bird coloration⁵.

We suggest the reason that female widowbirds prefer to nest in the territories of males with long feathers glued on to them, irrespective of total tail length, is that such males, through marginally more ponderous flight, are better decoys⁵ than normal or short-tailed males. Perhaps in a non-experimental context females choose to nest in the territories of males that happen to be good decoys through other non-genetic factors (for example, age, senility, damage). It does not follow, necessarily, that females also choose to mate with such males.

R. ROBIN BAKER

*Department of Zoology,
University of Manchester,
Manchester M13 9PL, UK*

G. A. PARKER

*Department of Zoology,
University of Liverpool,
Liverpool L69 3BX, UK*

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ANDERSSON REPLIES—Baker and Parker question the assumption (supported in other *Euplectes* species) that females tend to nest on the territory of the male with which they mate. If females mate randomly or prefer short-tailed males, but nest in territories of elongated males, the result is not evidence for Darwin's hypothesis. However, there seems no plausible reason to expect such a peculiar difference in female choice of nest site as opposed to mate.

In their other two points, Baker and Parker's criticism depends entirely on their unsupported assumption that the (small and nonsignificant; $P > 0.6$) difference between control males I and II did not arise from chance variation, but because elongated and cut-and-restored males through 'marginally more ponderous flight' attracted more females to nest (but not mate) on their territories. However, there is no indication that cut-and-restored males had more ponderous flight than the other control males. They showed almost identical changes in mean rates of flight display and attack after manipulation. Moreover, because feather vane was removed along the 1 cm joint to avoid vane overlap, the operation lightened each cut-and-restored feather by ~0.5 mg, including glue. The two parts of the feather were carefully aligned, and two specialists on bird flight, Drs Ulla and

Åke Norberg, on inspection considered any noticeable effect on the bird's flight performance as highly unlikely. The two control groups should therefore represent one single category from an aerodynamic as well as from the females' point of view. Their difference of 1 cm compared with a total tail length of ~50 cm would seem too small for females to notice; the difference to the other two groups was ~25 cm. It is therefore extremely unlikely that the cut-and-glue operation rather than changes in male tail length caused the significant trend in the result. Further, because cut-and-restored males were more successful than 'shortened' males, there is a hint that females preferred longer tails also within the normal range of lengths, contrary to the claim of Baker and Parker.

They favour their own predation hypothesis⁵, and suggest that females chose to nest on the territories of the best 'decoy' males, most likely to draw predators to themselves and hence to reduce the risk for female and young. But attraction of predators to the territory might just as well raise the risk also for the female and young. This alternative explanation therefore seems logically doubtful, in addition to being based on very unrealistic assumptions about differences between the control groups.

I therefore think it is most reasonable to interpret the trend of higher male success with increased tail length after manipulation as evidence that tail length influenced female choice as suggested by the Darwin–Fisher theory. However, further experiments are desirable in this or other species with similar ornaments and mating system, such as whydahs, peacocks and other pheasants.

I thank Frank Götmark and Åke Norberg for helpful comments.

MALTE ANDERSSON

*Department of Zoology,
University of Gothenburg,
PO Box 250 59,
S-400 31 Gothenburg, Sweden*

Matters Arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 500 words and the briefest of replies, to the effect that a point is taken, should be considered.

Self-conscious asides

Stuart Sutherland

Animal Thought. By Stephen Walker.
Routledge & Kegan Paul: 1983. Pp.437. £17.50, \$35.

FIFTEEN years ago, my interest in comparative psychology made it necessary to keep a number of different animals. A distinguished worker in artificial intelligence happened to visit me and asked: "Haven't you got anything better to do than keep a zoo?". Although at the time his remark seemed to lack a proper appreciation of the scientific spirit, the subsequent history of comparative psychology suggests that he may have had more sense than I credited him with.

At that time comparative psychologists were painstakingly measuring the performance of different groups of animals on a large number of learning tasks. They were trying to construct a two-by-two table on which the axes represented tasks and groups, and the cell entries represented performance. Apart from keeping themselves busy, they had two aims. First, by discovering sets of tasks in which any species that could perform one task could perform all the others, they hoped to infer the nature of the skill needed for all the tasks in the set. Second, by examining the neuroanatomical differences between the species that could and could not perform a given task, they hoped to learn about the brain mechanisms underlying successful performance. For several reasons this laborious approach to the problem of comparative intelligence proved a failure. It turned out that in many cases whether or not an animal could be trained to perform a task reflected not its own native wit, but the ingenuity of the experimenter in devising training appropriate to the animal's natural way of life. Moreover, the assumption that the same anatomical part of the brain, as defined by its evolutionary origin, has the same role in all species is false. Removing the hippocampus in a rat has very different effects on behaviour from those produced by removing it in man. Finally, there is often as wide a range of ability on a given task within a group as between groups.

Although Stephen Walker sets out to answer the question how far animal and human thought are similar, he is thoroughly aware of the difficulties that beset comparative psychology. He starts with a historical review of the opinions of earlier authorities, which is more interesting in its asides than in its main theme. Thus, it is of little consequence that Descartes thought animals (and machines) could never have knowledge or language, but it is of interest that Locke formulated the recently rediscovered concept of "working memory" (only a small number of ideas can be simultaneously manipu-

lated in consciousness) and that far from inventing the association of ideas as a general explanatory principle, he thought it was a thoroughly bad thing and was the explanation of the "madness, found in most men". Darwin was curiously soft on animals, ascribing to dogs a sense of humour and a belief in the supernatural. As to the behaviourists, they denied thought to both man and animals thus placing them on the same level.

Walker concludes that little reliance can be placed on the authorities and that one can only decide whether animals think like man by examining how far their behaviour and neuroanatomy are similar to our own. The idea that similarities in neuroanatomy are relevant to consciousness is not new. Walker quotes Charles Kingsley satirising Thomas Huxley who is made to declare that apes have "hippopotamus majors" in their brains just like men:

You may think that there are more important differences between you and an ape, such as being able to speak, and make machines, and know right from wrong, and say your prayers, and other little matters of that kind; but that is a child's fancy, my dear. Nothing is to be depended on but the great hippopotamus test.

Walker gives a clear account of comparative neuroanatomy, in which he displays a pleasing cynicism about the specious generalizations often drawn in that field. Much effort has been wasted measuring brain size and wholly useless equations have been produced, such as the formula governing the (approximate) ratio of brain weight to body weight, which for mammals and birds is $E = 0.07^{0.67} P$ (where E is the expected ratio and P is the body weight). Some intelligent species such as man, the crow and the porpoise have a brain weight considerably in excess of that predicted by this formula, but measuring brain size is no more likely to tell us anything significant about how the brain works than measuring the size of computers is to reveal the programs they can run. Moreover, the appearance of body weight in the ratio is curious. Birds, for obvious reasons, are very light, and so are sharks which have a cartilaginous skeleton and therefore turn in a high brain/body-weight ratio, although they are regarded as a primitive order of fish. Neanderthal man probably had a higher ratio than modern man, though he may of course have been more intelligent. As Walker remarks, since nobody has succeeded in assessing the intelligence of animals (or man come to that) objectively, there is no good evidence for an association

between intelligence and the brain/weight ratio.

He is equally scathing on the doctrine of encephalization of function, that is that in vertebrate evolution the locus of behavioural functions moves progressively forward in the brain, particularly into the forebrain, and neocortex. He points out that the hypothalamus is present in teleost fishes and can be regarded in all vertebrates as the head-ganglion of the autonomic system: its functions do not seem to have shifted. Moreover, although they only have a rudimentary cortex, birds display learning abilities in no way inferior to those of many mammals.

After an interesting but speculative chapter on the survival value of intelligence, Walker concludes with a survey of perception and memory in animals and an account of monkeys' use of knowledge and their ability to learn to use signs. He is somewhat preoccupied with the notion of conscious thought, and takes as evidence for it the finding that many animals can be trained to wait for a minute or more after having received a signal to respond, and can still make the correct reaction. But this surely does not imply that the animal has a conscious memory of the signal. Again, recent work on animal learning proves that animals do not learn merely to make responses on the basis of rewards and punishments — they learn what sequences of events occur, but this does not demonstrate that they have conscious expectancies, even though they act as though they do. Indeed one of the main problems with consciousness is that nobody has been able to suggest any evolutionary advantage for it. Men can learn and carry out highly skilled and complex motor patterns without being conscious of how the movements are executed and it is not apparent why taking certain decisions or using language should involve consciousness.

Walker is in fact concerned that consciousness may be bound up with language, in which case one would have to deny animals conscious thought. But we do not deny consciousness to infants and on her own account Helen Keller, who was deaf, dumb and blind, was conscious before she learned to communicate. Regardless of language, the similarity between human and animal behaviour and neuroanatomy surely justifies Walker in ascribing conscious thought to primates and other higher animals, but neither he nor anyone else is ever likely to penetrate the thoughts of a lamprey.

Animal Thought is a well-written and stimulating book, containing occasional flashes of wit. But it is interesting more for its *obiter dicta* and for its way of dealing with hallowed nonsense than for its main message which will surprise few readers.

Stuart Sutherland is Director of the Centre for Research on Perception and Cognition at the University of Sussex.

Petrologists at rock bottom

J.R. Cann

Petrology of the Ocean Floor.

By R. Hekinian.

Elsevier Scientific: 1982. Pp.393.

Dfl.200, \$111.

ROGER Hekinian has been in the business of ocean floor rocks since the early days of the 1960s. At that time geophysicists (who until then had been very pleased with themselves for all of their pioneering work on the physical properties of the ocean crust) realized that physical properties without rocks was like — well you can complete the cliché yourself. So ship time was found for us to start sampling the ocean floor properly. Hekinian and the rest of us were not the first, of course. The earliest rocks from the ocean floor came as a by-product of laying transatlantic cables in the 1860s, followed by the collections of *HMS Challenger*. After that the gap was long, punctuated by the occasional sponsored foray. The 1960s, though, saw a plate-tectonically inspired outburst of sampling, which flagged a bit in the middle of the following decade but is now in full swing again.

Hekinian has set out to document this activity fully. His book describes a very wide-ranging selection of the work on ocean floor rocks worldwide, concentrating particularly on areas in which Hekinian has worked in partnership with US, Canadian and now French marine geology teams. Some areas, such as the FAMOUS area, are given case-study status, and there the rocks are integrated with the other studies to paint an overall picture of hard-rock geology. Information from submersibles is used extensively where it is available, with special reference to the French work with the submersible *Cyana*. Tables of chemical analyses are frequent, and are of individual samples rather than of averages. I find these tables very necessary and useful, but I can understand how geophysicists often seem to regret letting us petrologists in on the marine game when we insist on throwing masses of numbers back at them, especially when it happens in lectures.

Hekinian gives a very broad coverage to his work. He includes rocks from mid-ocean ridges, transform faults, ocean basins, aseismic ridges, seamounts and ocean trenches, and he treats igneous, metamorphosed and weathered rocks as well as hydrothermal products such as the manganese oxides of warm springs and the sulphides of the spectacular black smokers. The breadth of coverage is such that the occasional gaps come as a shock. Thus Fig. 2.2, showing *Glomar Challenger* drill sites, is only complete up to early 1976, and does not even include the sites drilled when

Hekinian was co-chief scientist in 1977. Many more basement samples have been drilled since then. There is also a number of irritating misprints.

For me, the most valuable aspect is the abundance of new petrological and analytical work that Hekinian publishes here for the first time. I am sorry that he has not chosen to discuss in depth the significance he sees in the whole picture he has

compiled, but as he says perhaps what is needed most is a book that presents the facts first and foremost, leaving discussion to other places or other people. This book certainly makes a useful base on which to build. □

J.R. Cann is a Professor in the Department of Geology at the University of Newcastle upon Tyne.

Science meets law

Norman Carey

Patenting of Life Forms. Banbury Report 10.

Edited by D.W. Plant *et al.*

Cold Spring Harbor Laboratory: 1982.

Pp.336. \$65 (US), \$78 (elsewhere).

"RECOMBINANT DNA is a pest. No-one in my company can write a proposal for a new activity without some senior manager writing across it 'What will be the effect of recombinant DNA on this', and the thing goes into abeyance for months while they all think about it". Thus it was a few years ago that a rather frustrated scientific manager expressed to me the wish that all this excitement would settle down. There was, of course, a phase when the over-optimistic were predicting that recombinant DNA techniques would revolutionize everything in the applications of biological and medical research. The eventual outcome will probably be less extreme, but the impact on biological research as a whole of recombinant DNA and monoclonal antibody technology has undoubtedly been profound. Perhaps those senior managers were right to ask the questions.

One consequence of the flurry of commercial interest has been felt by academics, who suddenly found they had to handle a whole new set of relationships. One of these has been with patent lawyers. For this reason a group of scientists at Cold Spring Harbor initiated a conference at which patent lawyers from various parts of the profession could meet scientists, both academic and industrial, for mutual enlightenment. The *Patenting of Life Forms* is the printed record of the meeting, and makes interesting if rather heavy reading. Two themes run through the chapters of the book and the discussions which accompany them. The first is the complex question of the what and the how of patenting as applied to these new fields; the second is how academics should cope with all this commercial activity.

Like many similar meetings, the conference commenced with a series of papers on the basic science followed by explanations from the patent experts on the principles of patenting and their views on patents and the new genetics. The patenting process is often difficult to grasp and it

requires great rapport between an inventive scientist and his patent adviser to run the maze. Such rapport began to emerge at the meeting, but it was interspersed with periods of bewilderment, disbelief or disapproval. Not everything which is new is inventive, and the definitions of novelty and obviousness can be troublesome even without the need to apply the rules to a new field.

Much discussion on the patenting of microorganisms surrounds the famous Chakrabarty case, but much less has been made of the fact that there are those who think the affair may be a red herring, at least as far as recombinant DNA is concerned. There are many patent applications in this field which do not lay claim to a specific organism. Rather they claim DNA constructions which are operational when placed within a wide range of organisms. The uncertainty over whether this approach requires deposition of any organism, and what relationship the deposition debate bears to the question of enablement (a description sufficient to enable the invention to be repeated by an expert), may be the cause of much of the anticipated litigation. The embryonic twitchings of this issue are also to be found in the book.

In the few years in which I have been conversing with patent agents and lawyers on these topics I have found much to admire in the skill with which they can take the principles of patenting, together with examples from other fields, and arrive at a definition of an invention in molecular biology. The book reinforces that judgement and in addition provides two especially thought-provoking articles. In the first of these Kiley discusses the law of personal property and of title in the ownership of property. It is interesting to try and decide whether a cell line can be owned in the same way as a herd of cows or whether it more resembles a hive of bees. The question is by no means academic. The second article, by MacPherson, describes his experiences in the semiconductor industry in which on the whole patents give scant protection. The impression given here is that the fleet of foot, both scientifically and commercially, can find a niche in a rapidly moving field.

The second theme of the conference — the effect of the burst of commercial activity on the laboratories and individuals engaged in academic research — was not structured into the meeting but emerged

during many discussions. The pertinent technicality is that the information in a patent application must not be made public before the application is filed. At the conference worries emerged about universities with secret enclaves protecting trade secrets, but there were several other indications of the turmoil which the explosion of the field has caused. Doubtless there will be ill effects in some places, real or perceived, but it is hard to believe that all this striving will not in the end produce many benefits. Even so care must be taken that commercial interests do not distort the values of an academic laboratory.

Patenting of Life Forms is not the simplest way for molecular and cell biologists to learn about patenting or for patent agents to learn about the science. Nonetheless it does give all the basic information, and in the discussions exposes many misconceptions and starts to unravel a number of them. The book may in the end be of most interest to historians as an example of the evolving attitudes of scientists in the field. □

Norman Carey is Director of Research at Celltech, Slough, Berkshire.

Beginning on bacilli

Julius Marmur

Bacillus subtilis. The Molecular Biology of the Bacilli, Vol. 1.

Edited by David A. Dubnau.
Academic: 1982. \$44, £29.20.

IS A WHOLE volume on *Bacillus subtilis* and its phages justified? The short answer is "yes". David Dubnau, the editor, has selected some fine reviewers who handle the genetics, biochemistry, physiology and industrial aspects of the "second major prokaryotic subject for the study of molecular biology" with expertise at a level that will satisfy biologists ranging from graduate students to members of faculty. He has obviously done much to prevent serious overlap and has taken great pains to cross-reference information.

In the beginning, and always, there is *E. coli*. But, in relation to *E. coli*, *B. subtilis* has some different features and advantages — it is gram-positive, sporulates, is much more competent in DNA-mediated transformation and is sensitive to a wider variety of antibiotics; catabolite repression in *B. subtilis* does not involve cAMP, and the organism possesses an elaborate system for extracellular excretion and yields protoplasts that can fuse and revert efficiently; operons, if they exist, are rare; finally, *B. subtilis* is more readily handled for industrial exploitation than *E. coli*.

Most of the authors have covered their fields broadly, discussing information more fully than is usual in an *Annual*

Review article, presenting an historical perspective and, inevitably, emphasizing the comparative biology and biochemistry with that of *E. coli*. Comparisons have also been made to *S. pneumoniae* (transformation) and to other *Bacillus* species. Since most of the authors have spent a good deal of time working in the areas they cover, their own studies are discussed in considerable detail (in a few cases, perhaps too much) and references are up to date.

Dubnau's own contribution, "Genetic Transformation in *Bacillus subtilis*", is one of the highlights of the book. His hope "that the reader will be left with a clearer understanding of the molecular mechanisms involved in the uptake, processing and integration of transforming DNA" is fulfilled admirably. Plausible models and critical evaluations of results are helpful and useful. Dubnau explains why plasmid monomers fail to transform competent *B. subtilis* cells (making it difficult to shotgun clone homologous DNA into this organism). His discussions also have a direct bearing on other chapters.

Losick's chapter, "Sporulation Genes and their Regulation", is one of the most personalized and speculative presentations in the text. He proposes a positive transcriptional control model involving modifications of RNA polymerase promoter recognition components and interaction with spore gene encoded proteins to explain the developmental progression of "a developmentally ordered program of gene activation" in sporulation.

Examining this book, one wonders whether the information and concepts generated by work on *B. subtilis* will have a wider influence (as *E. coli* did) in the study of gene expression (which is the major emphasis in the book) in other organisms. The studies will certainly continue to affect research on other *Bacillus* species. An area where such influence might prove to have wider significance is based on the exciting investigations on developmental programming during spore development and temporal virulent phage DNA expression. In both cases changes in transcriptional patterns appear to be related to changes in RPase promoter recognition proteins and, in the case of sporulation, would also appear to involve the interaction of several gene products. Probably the most important role that the bacilli will play will result from their exploitation in the fermentation industry. The manipulation and improvement of strains need no longer be limited to a few traditional approaches.

This first volume to *The Molecular Biology of the Bacilli* is a very good beginning to the series. One looks forward with anticipation to subsequent volumes that will cover areas such as morphology, repair, chemotaxis, enzyme excretion and the molecular biology of other species. □

Julius Marmur is Professor of Biochemistry and Genetics at the Albert Einstein College of Medicine, New York.

Through the British Quaternary

Björn Kurtén

Pleistocene Vertebrates in the British Isles.

By A.J. Stuart.

Longman: 1982. Pp.212. £16.50, \$38.

THE study of Pleistocene vertebrates in Britain is beset with problems. Most of the fossil material (and much of the best) was collected long ago, with insufficient regard to stratigraphy — sometimes an entire cave was emptied in a few days. Furthermore, the collections are scattered in numerous local museums, and are in various states of curation. Working on such material, the student often finds himself with minimal returns as regards problems of fine stratigraphy, evolution at the population level and the like.

The haze is now being dispelled by a younger generation of palaeontologists. Many recent studies focus on the small mammals, of which stratigraphically localized and statistically respectable samples can be obtained relatively easily, using modern collecting techniques. Of special importance has been the great advance of Pleistocene palaeobotany in the British Isles, and vertebrate palaeontology at its best is now developed hand in glove with palaeobotany. The author of the present volume is one of the leading students in this area and his handsome book is a fine testimony to all of the work carried out in recent decades.

Although palaeobotany is an excellent climatic tool, the slow evolution of plant species makes it somewhat insensitive for biostratigraphical analysis (the same holds for palaeoentomology); by contrast, the rapidly evolving mammalian species may help to discern cases of repeated, essentially similar climatic cycles. The number of interglacials in the later Pleistocene, for instance, is disputed. Palaeobotanists distinguish three (the Hoxnian, Ipswichian and Flandrian), while comparison with the climatic record obtained from marine sediments suggests a greater number. This is currently the main controversy, and the author adheres to the three-interglacial scheme, although properly noting that it may become a straitjacket.

Cheaper fluid dynamics

Springer-Verlag have issued a paperback "study edition" of Joseph Pedlosky's *Geophysical Fluid Dynamics*. The new edition is a corrected reprint of the original hardcover edition of 1979, and costs DM63, \$26.30. *Hydrodynamic Stability* by P.G. Drazin and W.H. Reid (reviewed in *Nature* 294, 196; 1981) has also recently appeared in paper covers. Publisher is Cambridge University Press, price £12.50, \$24.95

The vertebrate species known from the British Quaternary are described with emphasis on the mammals, illustrating salient anatomical characters. A notable feature among the profuse and attractive pictures is a series of maps showing the present-day distributions of British Pleistocene species, giving an impression of the degree of distributional change since the Ice Age. The organization of the book, however, leaves something to be desired. The author's approach is to cover the same ground (the British Quaternary succession) from various viewpoints (stratigraphy, climatic events, palaeogeography, vegetational history, and faunal history) which seems unduly repetitious. The German

tradition with its *Allgemeiner Teil* and *Spezieller Teil* has much to recommend it.

Despite occasional idiosyncrasies (refusal to accept the identification of Merck's rhino in Britain) and errors (the interpretation of proboscidean phylogeny is obsolete), the book is timely, and will remain an important standard work for Pleistocene workers. It is also recommended reading for the numerous amateur students in the field, for whose benefit the appendix on the collection and preservation of fossils might well have been expanded into a full chapter. □

Björn Kurtén is Professor of Palaeontology at the University of Helsinki.

Clothing the chemist

Jon McCleverty

Inorganic Chemistry: A Modern Introduction.

By Therald Moeller.

Wiley: 1982. Pp.830. £31, \$46.50.

IN TEACHING inorganic chemistry one faces a dilemma — whether to deliver the subject in a factual way based on the Periodic Table, or to present it as a series of topics. Practitioners of the first approach feel that students should know facts, supported by theories. The problem is then that the facts can be overwhelming and, in any case, there just isn't time to deal satisfactorily with 105 or so elements. Many of us now take the second route, choosing our topics partly on academic grounds and partly in terms of the constraints of time and resources. One hopes that, towards the end of a such a course, the student will be able to put together, from all the various garments we have woven, an adequate fit not complete suit of inorganic clothes.

In *Inorganic Chemistry: A Modern Introduction* Moeller has opted for the "topic" approach. There are plenty of facts carefully interwoven around the central threads of theories and contemporary rationalizations. A most attractive feature is the presentation of the state of our inorganic art alongside its historical development, the relationship between old and new being well-balanced and informative. The sections dealing with coordination chemistry, which are preceded by a succinct description of the applications of group theory, are particularly well-written. There are excellent discussions of various aspects of solid-state chemistry (including metals) and of physical methods for the determination of molecular structure (there are even tables of fingerprint regions in vibrational spectra!). Such material is frequently omitted from other prominent texts. Every chapter includes a very useful list of general references which have been drawn from the international literature.

For us in Britain, one difficulty with textbooks which originate across the Atlantic is how the subject matter relates to courses based on our three-to-four year first degree. Moeller seems to have overcome this problem by including almost everything that nearly all inorganic chemists would agree should form the core studies of their subject. What is excluded (e.g. a detailed discussion of the M-C bond) is arguably inessential for a basic course.

I really like this book — it is immensely readable, and is a splendid teaching text for the early and middle years of a modern course. I am critical, however, of the hopeless inadequacy of the contents list and of the just acceptable subject index (is the author index really necessary?). While the book certainly matches almost exactly the requirements of the inorganic chemists in my own institution, I fear it will not reach the recommended list purely and simply because of its price vis-à-vis the competition. □

Jon McCleverty is Professor of Inorganic Chemistry at the University of Birmingham.

Sins of inclusion

Franklin W. Stahl

Genetic Recombination: Understanding the Mechanisms.

By Harold L.K. Whitehouse.

Wiley: 1982. Pp.415. £23.75, \$49.95.

HAROLD Whitehouse has judiciously divided his subject into six major areas: transformation, single-stranded phages, phage T4, phage λ , *E. coli* and eukaryotes. Within each of these areas he has concisely yet lucidly surveyed the salient experimental and conceptual contributions, more or less chronologically. His book is a fine accomplishment and represents a worthy product of a long and intense effort to understand God's mechanisms for shuffling the genetic deck.

Whitehouse surveys each of the areas thoroughly and with a fair mind. Each of these valuable qualities nonetheless carries with it responsibilities and dangers. First, the surveys are so thorough that they may become *de facto* official histories. This could mean that the few inaccurate attributions might lead to rather profound injustices; for instance, our knowledge of the delightful ability of the *recBC* gene product to wiggle its way along between the chains of a DNA duplex, separating those chains as it moves, is attributed to authors whose work, while interesting, was supplementary to the uncited work of Rosamond *et al.* (*J. Biol. Chem.* **254**, 8646; 1979).

Second, the surveys are so fair-minded that they are sometimes in danger of being uncritical. Out of apparent deference to historical accuracy, interpretations are presented that, though compelling in their original context, can now be seen to be based on misunderstanding. For instance, the phage mating theory of Visconti and Delbruck (*Genetics* **38**, 5; 1953) accounted for non-localized negative interference by supposing that individual phage genomes differ in the numbers of "pairwise matings" they have enjoyed and that exchanges occur without interference in these individual matings. Subsequent work, however, has revealed that the central idea of the mating theory, for T-even phage, is wrong. As Drake has shown (*Proc. Natn. Acad. Sci.* **58**, 962; 1967), there are no pairwise matings, and negative interference results from miscellaneous heterogeneities plus the circularity of the linkage map (F.W. Stahl and C. M. Steinberg *Genetics* **50**, 531; 1964).

If the present text has a theme, it is that the complexities of recombination, though sometimes daunting, are pretty universal, so that they apply even to widely unrelated groups of organisms. In what I construe as an effort to be true to this message, Whitehouse backs the view that correction of mismatches in stretches of hybrid DNA is the sole mechanism of gene conversion. He acknowledges one dissident voice but puts it down as he did previously in his article with Holliday (*Molec. Gen. Genet.* **107**, 85; 1970). I had hoped that the forceful demonstration by Orr-Weaver *et al.* (*Proc. Natn. Acad. Sci.* **78**, 6354; 1981) of gene conversion by double strand gap repair would have liberalized Whitehouse's view. But, perhaps it has, and *Genetic Recombination* might have been in press when the work of Orr-Weaver and her colleagues appeared.

Enough harping. This book is required reading for all who would work and write in the field of genetic recombination mechanisms. It will be an invaluable aid to us in avoiding sins of omission. □

Franklin W. Stahl is Professor of Biology and Research Associate in the Institute of Molecular Biology, University of Oregon. He is author of Genetic Recombination: Thinking about it in Phage and Fungi (W. H. Freeman, 1979).

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Appointments

The following have been elected Fellows of the Royal Society: **Dr M.J. Aitken** (University Research Laboratory for Archaeology and History of Art, Oxford, UK); **Dr P.P.G. Bateson** (Sub-Department of Animal Behaviour, University of Cambridge, UK); **Professor S. Chandrasekhar** (Liquid Crystal Laboratory, Raman Research Institute, India); **Professor E.C.D. Cocking** (University of Nottingham, UK); **Professor P. Deslongchamps** (Université de Sherbrooke, Canada); **Professor W.W. Douglas** (Yale University School of Medicine, USA); **Professor R.J. Ellis** (University of Warwick, UK); **Professor M.A. Ferguson-Smith** (Glasgow and West of Scotland Genetic Advisory Centre, UK); **Professor A.R. Fersht** (Imperial College of Science and Technology, London, UK); **Professor W.A. Gambling** (University of Southampton, UK); **Professor I.G. Gass** (Open University, UK); **Professor I.R. Gibbons** (University of Hawaii, USA); **Professor G.W. Gray** (University of Hull, UK); **Professor R.W. Guillery** (University of Chicago, USA); **Dr R. Henderson** (Medical Research Council (MRC) Laboratory, Cambridge, UK); **Professor P.W. Higgs** (University of Edinburgh, UK); **Professor C. Hooley** (University of Wales, UK); **Dr A.T. James CBE** (Unilever Research Colworth Laboratory, Bedford, UK); **Dr P.A. Lawrence** (MRC Laboratory of Molecular Biology, Cambridge, UK); **Dr J.D. Lawson** (Science and Engineering Research Council (SERC) Rutherford Appleton Laboratory, Didcot, UK); **Professor G. Lusztig** (Massachusetts Institute of Technology, USA); **Professor C.D. Marsden** (Kings College Hospital Medical School, UK); **Professor D. Metcalf** (The Walter and Eliza Hall Institute for Medical Research, Melbourne, Australia); **Professor R.K. O'Nions** (University of Cambridge, UK); **Professor E.G.S. Paige** (University of Oxford, UK); **Dr M. Pepper** (Cavendish Laboratory, Cambridge, UK); **Dr E.J.C. Polge** (Agricultural Research Council (ARC) Institute of Animal Physiology, Cambridge, UK); **Professor M.J.D. Powell** (University of Cambridge, UK); **Professor P.J. Randle** (University of Oxford, UK); **Professor I.M. Roitt** (Middlesex Hospital Medical School, UK); **Professor A. McLeod Sargeson** (Australian National University); **Dr D.W. Sciama** (University of Oxford, UK); **Professor I.N. Sneddon OBE** (University of Glasgow, UK); **Dr E.M. Southern** (MRC Mammalian Genome Unit, Edinburgh, UK); **Professor D.B. Spalding** (Imperial College of Science and Technology, London, UK); **Dr P.N.T. Unwin** (Stanford University, USA); **Professor I.M. Ward** (University of Leeds, UK); **Professor F.J. Weinberg** (Imperial College of Science and

Technology, London, UK); **Professor J.H. Westcott** (Imperial College of Science and Technology, London, UK); and **Dr D.H. Williams** (University of Cambridge, UK).

The Royal Society of Edinburgh has elected **Professor Malcolm Peaker** and **Dr William Banks** of the Hannah Research Institute to fellowship.

Geoffrey Caston, who has been secretary general of the Committee of Vice-Chancellors and Principals since 1979 and who was formerly registrar of the University of Oxford, has been appointed Vice-Chancellor of the University of the South Pacific.

The current professor of epidemiology and director of the MRC Environmental Epidemiology Unit at the University of Southampton, **Sir Henry Yellowlees**, has been appointed chief medical officer of the British Department of Health and Social Security with effect from October 1983.

The University of Southampton, UK, has appointed **Sir Bernard Mill**, a former chairman of the John Lewis Partnership, to be pro-chancellor of the university. **Lord Shackleton**, **J. Fairclough** and **D. Scouller** have also been appointed members of the University Council.

The Open University of the United Kingdom has announced two new appointments. **David Grugeon** has been made the pro-vice-chancellor of the university, and **Michael Bradford** has taken up a three year appointment as director of regional academic services.

The World Health Organisation (WHO) has appointed **Dr Serguei Litinov**, previously deputy chief of the external relations board, the assistant director of WHO.

Dr Austin Hughes has been appointed director of the National Vision Research Institute and a professor of the University of Melbourne, Australia.

Three new appointments have been made to the British University Grants Committee. **John Cannon**, professor of history at the University of Newcastle upon Tyne and **William Semple** director of education for Lothian Regional Council have already joined the committee, and the appointment of **Sir Peter Baxendell**, chairman of Shell Transport and Trading Company will take effect from 1 July 1983.

Maggie Siggins has been appointed to the Max Bell Chair of Journalism at the University of Regina's School of Journalism and Communications.

The chairman of Gerrard and National Discount, **Roger Gibbs**, has been appointed a trustee of the Wellcome Trust.

Sir Alistair Frame has been elected chairman of the Australian Council of Mining and Metallurgical Institutions.

The senior lecturer in the department of haematology of the Welsh National School of Medicine **Dr M. Wormwood** has been awarded a readership in haematology.

Awards

Harkness Fellowships for study in the United States of America have been awarded by the Commonwealth Fund of New York to **S.M.St.J. Alexander** (Boston Consulting Group, London) Business Administration; **J. Bone** (Queens' College, University of Cambridge) International Relations/Journalism; **A.M. Brandenburger** (Trinity College, University of Cambridge) Economics; **W.R. Breckon** (Mathematics Institute, University of Warwick) Mathematics; **R.J. Broadbent** (H.M. Treasury, London) Industrial Relations; **T.F. Chapman** (Christ's College, University of Cambridge) Anthropology; **Helena Cook** (Coward Chance, Brussels) Law; **Daisy Goodwin** (Trinity College, University of Cambridge) Film Studies; **Alexandra Jones** (New College, University of Oxford) Public Policy; **J.P. Morgan** (Artist) Art History/Painting; **Lorna Parker** (Kleinwort Benson Ltd., London) Business Administration; **Suzanne Raitt** (Jesus College, University of Cambridge) English Literature; **A. Rees** (Exeter College, University of Oxford) Neurophysiology; **N.G.J. Richards** (Trinity College, University of Cambridge) Chemistry; **C.H. Smith** (Artist and part-time lecturer) Painting; **R.R.R. Smith** (Magdalen College, University of Oxford) Classical Studies; **R.P. Tomlinson** (Christ's College, University of Cambridge) History; **B.A. Trimmer** (Department of Zoology, University of Cambridge) Neurobiology; **C.H.W. Upwards** (Ensam, Paris) Industrial Engineering.

Leon Lederman, director of the US Department of Energy's Fermi National Accelerator Laboratory at Batavia, Illinois (Fermilab) is to share the \$100,000 Wolf Prize given by the Wolf Foundation of Herzliya Israel with **Martin Perl** of the Stanford Linear Accelerator Centre (SLAC).

The Kaj Linderstrom-Lang prize for 1983 has been awarded to **Professor Harold Scheraga** of the department of chemistry, Cornell University for his contributions to understanding the interactions which determine the folding of proteins in aqueous solution.

The first four recipients of the new Royal Society of Edinburgh Bicentenary Medal are the **Lord Balerno of Currie, Emeritus Professor Neil Campbell, Dr Anthony Richie and Dr Robert Schlapp.**

The Society for Drug Research Award for Drug Discovery has been given to **Peter Doyle** in recognition of his contribution over many years to the development of new pharmaceutical products, and in particular the discovery and use of beta-lactam antibiotics.

Recipients of the 1982 Wolf Foundation Prizes announced last month are: **Wendell Roelofs** (Cornell University) Agriculture; **Hassler Whitney** (Institute for Advanced Study, Princeton) and **Mark Krein** (Institute of Physical Chemistry of the Ukrainian S.S.R. Academy of Sciences) Mathematics; **John Polanyi** (University of Toronto) and **George Pimentel** (University of California) Chemistry; **Leon Lederman** (Fermi National Accelerator Laboratory) and **Martin Perl** (Stanford Linear Accelerator Center) Physics; **Jean-Pierre Changeux** (Institut Pasteur) **Solomon Snyder** (Johns Hopkins University Medical School) and **Sir James Black** (Wellcome Research Laboratories) Medicine; **Vladimir Horowitz**; **Olivier Messiaen**, and **Joseph Tal** Art (this year Music).

The Grand Cross of Merit of the Federal Order of the Federal Republic of Germany has been presented to **Dr Heinz Gotze** for his contributions to science and for his involvement in publishing and the book trade.

The 1982 SmithKline Foundation biennial prize for research in clinical pharmacology has been awarded to **John Feely** a lecturer in pharmacology and therapeutics at the University of Dundee and honorary senior medical registrar at Ninewells Hospital and Medical School, Dundee.

The Dautrebande prize for physiopathology has been given to **Professor Hugues** from London and **Professor Kosterlitz** from Aberdeen. Professors Hugues and Kosterlitz have been chosen for their contributions to understanding the biochemistry of the neurotransmitter cerebral. Applications for the 1985 Dautrebande prize of 2 million Belgian francs should be set to Dr Stalport, President, 35 chaussée de Liège, 5200 Huy, Belgium.

Balliol College, Oxford is inviting applications for the first Julian Huxley junior research fellowship. It will be tenable for three years from October 1983 by anyone pursuing a project of high promise in biological sciences (fields related to evolution and genetics). Further information: Mrs C. Phillips, The New Grounds, Slimbridge, Gloucester GL2 7BT, UK.

The Filtration Society of Great Britain are offering two travel and study awards in 1983, each worth £400. Application forms are now available from the Filtration Society, c/o Department of Chemical Engineering, University of Technology, Loughborough, Leics LE11 3TD, UK.

Applications are invited for the Clarke Institute of Psychiatry Annual Research Fund Award of C\$1,000 for research in psychiatry in Canada. The prize will be awarded to a clinical or basic scientist who has published a report or dissertation on outstanding research within the field of mental health, which must have been conducted during the preceding year. The research should have been carried out in Canada by a resident of that country. Applicants should forward four copies of papers to the Secretary of the Research Fund Committee, Board of Trustees of the Clarke Institute of Psychiatry, 250 College Street, Toronto, Ontario, Canada M5T 1R8.

The British Laboratory Ware Association has set up the new Labtechnology Awards, which will be made for products showing a degree of innovation which has made a significant contribution to the progress of scientific laboratory technology. The first, second and third prize winners will receive £500, £400 and £150 respectively and applications should be submitted on an official entry form, which is available from BLWA Labtechnology Awards, 30-32 Worpole Road, London SW19, UK).

Meetings

6-12 August, **Annual Meeting of the Electron Microscopy Society of America**, Phoenix (Dr P. Calarco, Department of Anatomy, University of California, San Francisco, Medical Centre, San Francisco, CA 94143, USA).

7-12 August, **Joint Meeting of the Herpetologists' League and the Society for the Study of Amphibians and Reptiles**, Salt Lake City (Professor J.M. Legler, Department of Biology, University of Utah, Salt Lake City, UT 84112, USA).

8-24 August, **International Training Course on Insect Growth, Development and Behaviour — Insect Chemoreception**, Nairobi (Dr L.O. Abe, Principal Training Officer, The International Centre of Insect Physiology and Ecology (ICIPE), PO Box 30772, Nairobi, Kenya).

14-19 August, **1983 Solar World Congress and the International Solar Energy Exhibition**, Perth (Mr B. Wood, Congress Coordinator, 13 Howard Street, Perth, Western Australia 6000).

14-20 August, **International Symposium on Anaerobic Digestion**, Boston (Third International Symposium on Anaerobic Digestion, 99 Erie Street, Cambridge MA 02139, USA).

15-18 August, **National Symposium on Fracture Mechanics**, Columbus, Ohio (Dr M.G. Kanninen, Battelle Columbus Laboratories, 505 King Avenue, Columbus, OH 43201, USA).

15-27 August, **General Assembly of the International Association of Seismology and Physics of the Earth's Interior**, Hamburg, West Germany (Dr R.D. Adams, Secretary General, IASPEI, International Seismological Centre, Newbury, Berks RG13 1LZ, UK).

17-20 August, **International Symposium on Plecoptera**, Toulouse, France (Professor C. Berthélemy, Laboratoire de Zoologie, Université Paul Sabatier, 118 route de Narbonne, F. 31062 Toulouse, France).

17-20 August, **International Congress of Reproductive Immunology**, Kyoto, Japan (Professor S. Isojima, Department of Obstetrics and Gynaecology, Hyogo Medical College, 1-1 Mukogawacho, Nishinomiya 663, Japan).

21-26 August, **International Conference on Chemistry, Education and Society**, Montpellier, France (Dr D. Cros, 7th International Conference on Chemical Education, Laboratoire des interactions moléculaires, Université des Sciences et Techniques du Languedoc, Place Eugene Bataillon, 34060 Montpellier, France).

21-27 August, **International Congress of Immunology**, Kyoto, Japan (Dr T. Kishimoto, General Secretary, 5th International Congress of Immunology, The Third Department of Internal Medicine, Osaka University Hospital, Fukushima, Osaka 553, Japan).

22 August-3 September, **International Cosmic Ray Conference**, Bangalore, India (Conference Secretariat, Tata Institute of Fundamental Research, Homi Bhabha Road, Bombay 400 005, India).

22-24 August, **International Symposium on Mechanisms of Vasodilation**, Sydney (Scientific Secretariat, Mrs J. Beckman, 942C Guggenheim Building, Mayo Clinic, Rochester, MN 55095, USA).

22-26 August, **NATO Conference on Ageing and Technological Advances**, University of South California (Dr J. Birren, Executive Director, Andrus Gerontology Centre, University of Southern California MC-0191, Los Angeles, CA 90089-0191, USA).

22-26 August, **International Conference on Biometeorology**, New Delhi (Professor S.C. Pandeya, Head of the Department of Biosciences, Saurashtra University, Rajkot 360 005, India).

29 August-2 September, **World Congress on Nuclear Medicine and Biology**, Paris (Congres Services, 1, rue Jules Lefebvre, F-75009 Paris, France).

29 August-6 September, **International Ethological Conference**, Brisbane (Conference Secretary, 18th International Ethological Conference, Animal Behaviour Unit, University of Queensland, St. Lucia, Q 4067, Australia).